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Chapter One

Introduction



Introduction Contents

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1.1 Steroidal Compounds: An Overview

Steroids are a class of natural or synthetic organic compounds, characterised by a molecular structure of 17 carbon atoms constituting 4 cycloalkane ring systems fused together in a certain fashion (Figure 1.1).¹⁻⁹ Steroids have important biological, chemical and medicinal roles, ranging from pain relief to promoting growth of muscles and bones. However in humans, most steroids function as hormones. That such diverse physiological functions and effects should be exhibited by steroids, all of which are synthesized by essentially the same central biosynthetic pathway, is a remarkable example of biological economy.¹⁰

The most abundant steroids are the sterols, a subclass of steroid compounds which contain alcohol functionality. Typically, cholesterol is the most familiar sterol in public awareness, due to the now widespread knowledge that excess cholesterol is a risk factor for cardiovascular disease. For this reason much attention has been focussed on cholesterol lowering substances, both in the academic community and in the media.^{11,12} However, sterols have a wide range of functions in animals¹³ such as acting as a structural component of membranes, as sex hormones¹⁴ and in pheromone signalling.¹⁵

Phytosterols are the sterols which are synthesised only in plant materials, and which mammals must obtain *via* dietary sources.¹⁶ They are commonly referred to as plant sterols, and they are known for their cholesterol-lowering properties when they are consumed *via* phytosterol enriched food substances.^{17,18} The ability of phytosterols to lower cholesterol levels in humans is well documented,¹⁹ however there is no evidence to show that they exhibit any beneficial effect on cardiovascular disease or overall mortality rate.^{20,21}

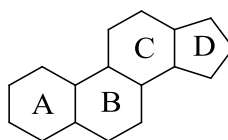


Figure 1.1 The core steroidal nucleus

1.2 Lanosterol

Lanosterol **1** is a naturally occurring steroid found in sheep wool.²² Its structure comprises the core four rings of the steroidal nucleus and an unsaturated alkyl side chain at C-17, as shown, with numbering Scheme, in Figure 1.2. Functionality of note in the structure of lanosterol **1** are the alkenes and the alcohol. Two alkene bonds are present in the compound, one highly sterically hindered double bond at C-8,9, and one in the side chain at C-24,25, which is less sterically hindered. The “sterol” component of the lanosterol **1** nomenclature arises from the alcohol moiety present in the structure at C-3.

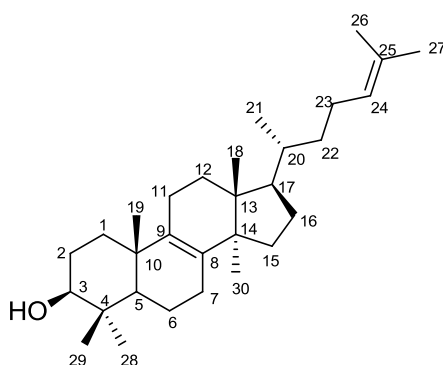


Figure 1.2 Lanosterol **1** numbering scheme

The six membered rings in lanosterol **1** exist in chair conformations, with their attached methyl groups orientated in axial and equatorial positions accordingly, as shown in Figure 1.3.²³ The orientation of these methyl groups is crucial in terms of the chemical reactivity of lanosterol, and in particular the stereochemical outcomes of reactions at the C-8,9 alkene, which will be discussed in due course.

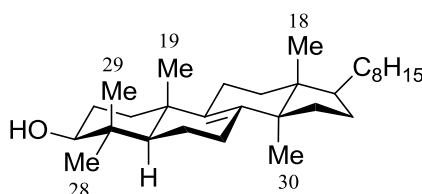


Figure 1.3

Lanosterol, being the precursor of cholesterol, is also the precursor for the steroid hormones oestrogens androgens and progesterones.^{24,25} Steroid hormones (or sterones) are crucial substances for the correct function of the body. They mediate a wide variety of vital physiological functions ranging from anti-inflammatory agents to regulating events during pregnancy.

As well as being naturally present in the body, lanosterol is applied topically due to its presence in a variety of cosmetic formulations. Various hair conditioners, skin moisturisers and lipsticks contain lanosterol as a moisture retaining natural animal fat.²⁶⁻²⁸

1.3 Cholesterol

Cholesterol **2** is a sterol produced naturally in the human body and also consumed *via* dietary fats.²⁹⁻³² The structure of cholesterol **2** is similar to that of lanosterol. The four ring steroidal skeleton of cholesterol **2** differs from that of lanosterol in the position of the alkene bond at C-5,6 and also the absence of the C-28, C-29 and C-30 methyl groups, as highlighted in Figure 1.4. The side chain of cholesterol **2** contains the same number of carbon atoms with the same connectivity as lanosterol, however the C-24,25 bond is saturated in cholesterol **2**.³³

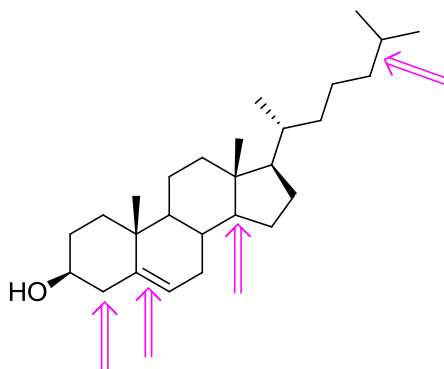


Figure 1.4 Cholesterol **2** structure and points of differentiation from lanosterol

The six membered steroidal rings of cholesterol **2** adopt the same chair conformation as lanosterol. However, when the structure of cholesterol is viewed in perspective, it is clear that

the sites of reactivity are far less sterically hindered in cholesterol, due to the absence of three methyl groups and the repositioning of the alkene bond, as can be seen in Figure 1.5.

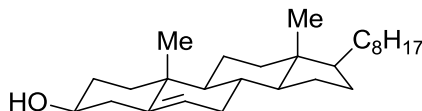


Figure 1.5

1.4 Stigmasterol

Stigmasterol **3** is a naturally occurring phytosterol which is found in the plant fats or oils of soybean, calabar bean and rape seed.^{1,2,34-37} It is also found in medicinal herbs including American Ginseng, along with various vegetables, nuts and seeds. As phytosterols are essential components of plant cells, they are naturally present in the human diet.³⁸

As discussed earlier in the overview section, phytosterols have been widely publicised due to their cholesterol lowering abilities. Although cholesterol is not a phytosterol, it is closely structurally related to common dietary phytosterols such as campesterol **4**, sitosterol **5**, and stigmasterol **3** (Figure 1.6). It is generally believed that the cholesterol-lowering properties of phytosterols are therefore centred on metabolic events shared by the two steroids. For example, one way phytosterols lower serum LDL cholesterol is by disrupting intestinal cholesterol solubilization into micelles.³⁹

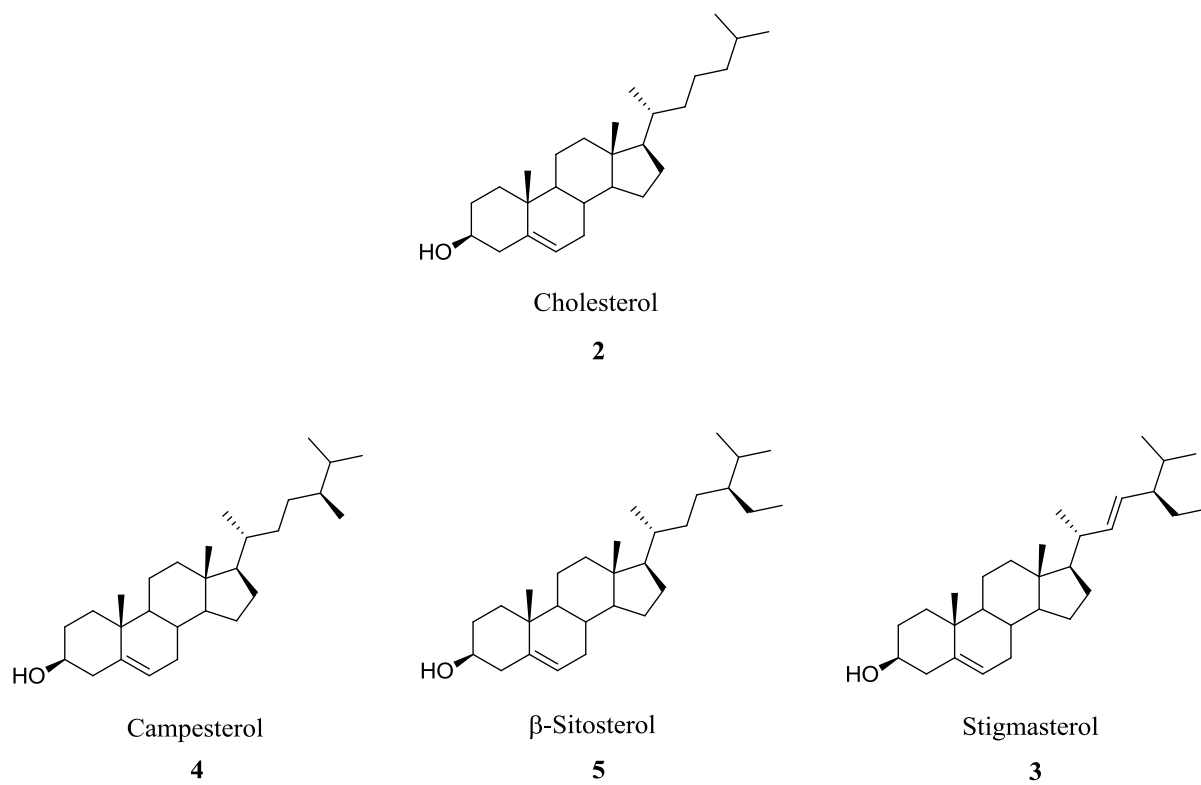


Figure 1.6

1.5 Biosynthesis of Lanosterol

Lanosterol is biosynthesised *via* the C₃₀ compound squalene **6**, also a natural substance, found in abundance in shark liver oil.^{40,41} Squalene **6** contains two C₁₅ farnesyl **7** moieties, joined together in a tail-to-tail fashion, as opposed to the head-to-tail fashion observed for smaller terpenoids. The mechanism by which these two C₁₅ units condense proceeds *via* the formation of the cyclopropane containing compound presqualene pyrophosphate **8**.

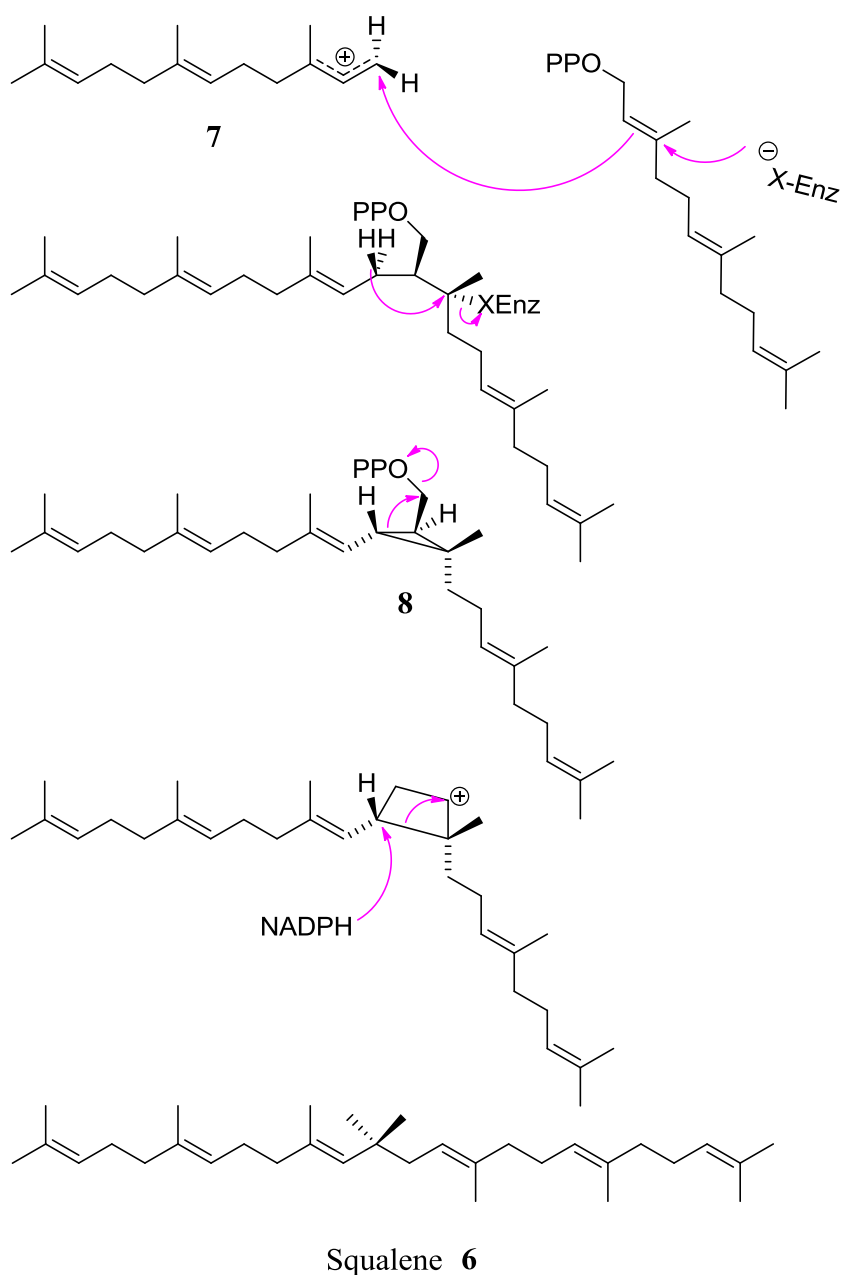


Figure 1.7

Squalene **6** is selectively epoxidised to form 2,3-oxidosqualene **9** which then cyclises in an apparently concerted fashion to yield lanosterol **1** (Figure 1.8).⁴² The catalysts involved in this process are squalene epoxidase and epoxysqualene cyclase.

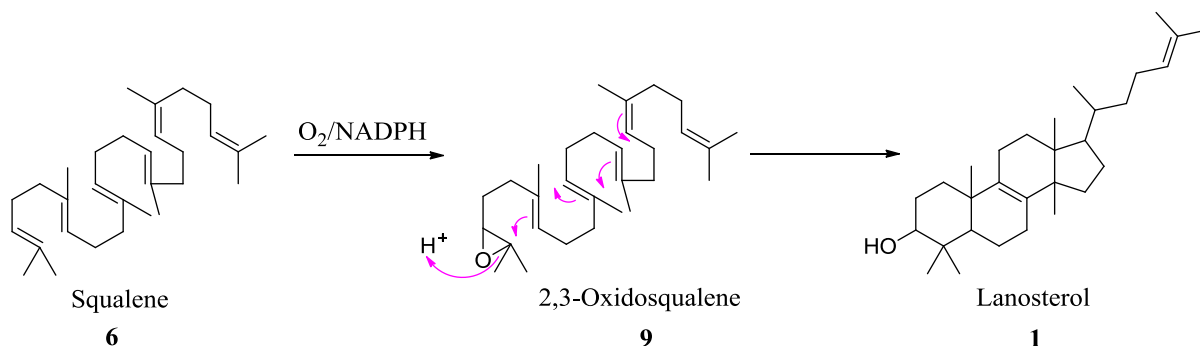
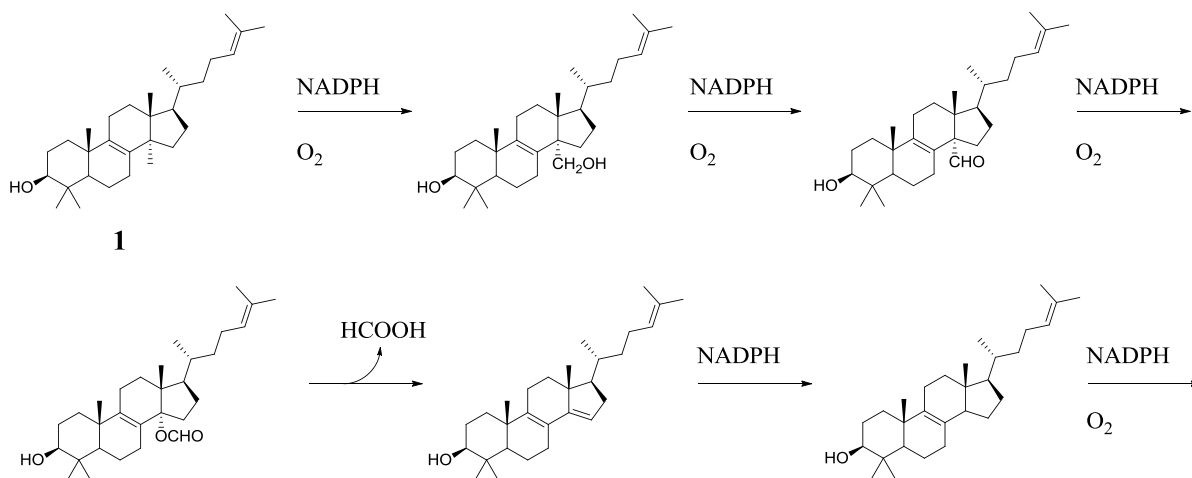
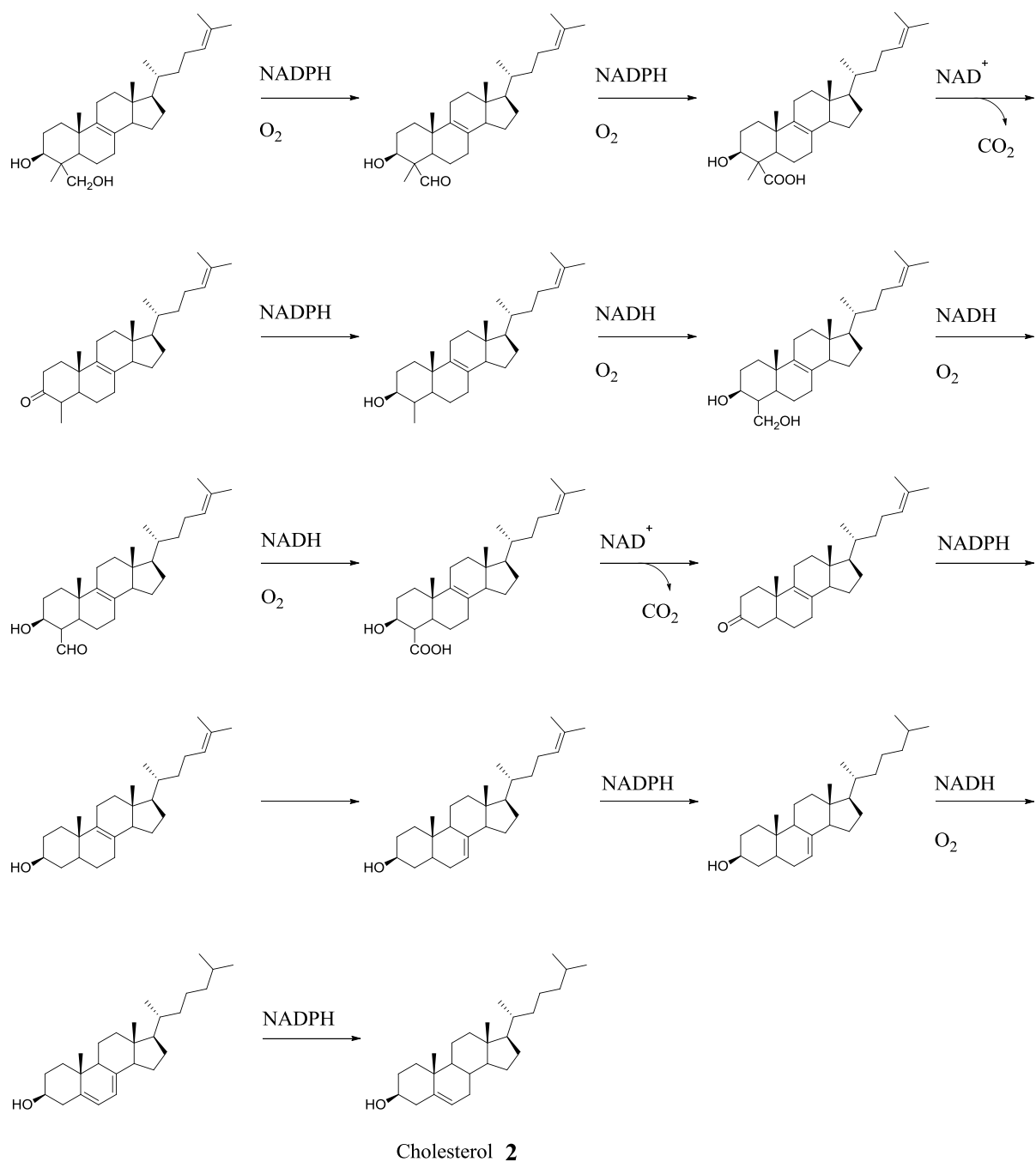


Figure 1.8

1.6 Biosynthesis of Cholesterol

Lanosterol **1** is the precursor for cholesterol **2** biosynthesis, an intricate nineteen step process which utilises nine different enzymes, (Scheme 1.1).¹⁰ All animal cells manufacture cholesterol, with relative production rates varying depending on cell type and organ function. The rate limiting step in the overall biosynthesis of cholesterol is catalysed by 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) which catalyses production of mevalonate from HMG-CoA. Therefore, this enzyme is a prime target for inhibition, if cholesterol production is to be reduced.¹²





Scheme 1.1

The biosynthesis of cholesterol from lanosterol is similar to other metabolic pathways, in that the enzymes involved catalyse a series of reduction and oxidation reactions, along with carbon-carbon bond cleavage, epimerisation, isomerisation and dehydrogenation processes. Removal of three methyl groups from lanosterol requires carbon-carbon bond cleavage, a process which is accomplished by oxidation of the methyl groups and their subsequent elimination as formic acid or carbon dioxide.

1.7 Biosynthesis of Stigmasterol

The isoprene units needed for the biosynthesis of stigmasterol **3** are supplied exclusively from the mevalonate pathway. The biosynthesis follows a similar pattern to that of lanosterol, with a squalene-like compound being incorporated (Figure 1.9).⁴³

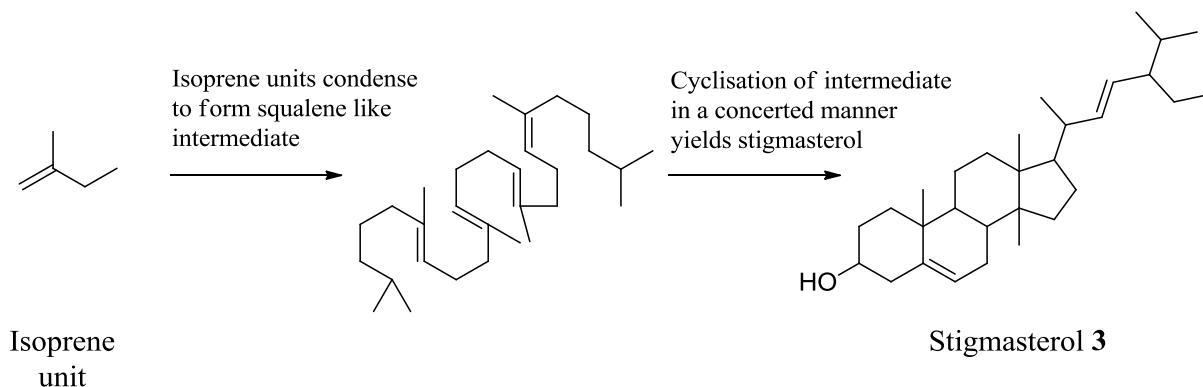


Figure 1.9

1.8 Isolation of Lanostane Compounds from Natural Sources

While there has been a long standing high level of interest in the biological effects of cholesterol and the phytosterols, interest in lanosterol and its oxidation products has been a much more recent development. The isolation of lanostane type compounds from various natural sources has been recently documented.⁴⁴⁻⁴⁸

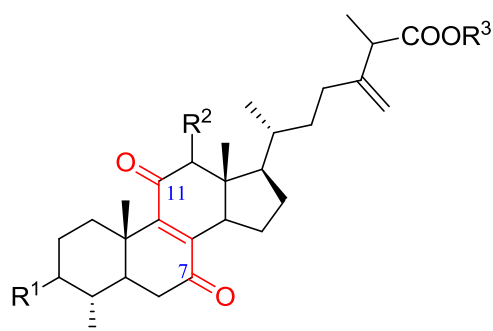
An example of a natural source of lanostane compounds which has attracted much pharmaceutical attention, is the medicinal mushroom *Antrodia camphorata*, a Ganoderma-like parasitic fungus which belongs to the Polyporaceae Basidiomycotina family. It is a rare species which grows only on the endemic perennial Bull Camphor tree at an altitude of 450 – 2000 m in the Taiwanese mountains.⁴⁹ The medicinal value of this mushroom has long been known, as it has been used in traditional Chinese remedies for abdominal pain, diarrhoea, hypertension and skin itching. Recently, *Antrodia camphorata* has attracted the attention of the pharmaceutical industry due to its potential anti-tumour activity. It has also displayed other biological activities of pharmaceutical interest, including antihepatotoxic,⁵⁰

antihypertensive⁵¹ anti-inflammatory⁵² and antioxidant⁵³ activities. More recently *A. camphorata* has been reported to display antiproliferation, cytotoxic, or DNA topoisomerase-inhibiting effects against cancer cells.^{54,55}



Figure 1.10 Ganoderma type fungus

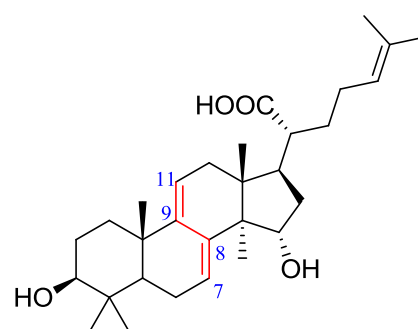
The chemical constituents of the *Antrodia camphorata* include diterpenoids, triterpenoids, sesquiterpene lactone and benzenoids. The major phytochemical constituents of medicinal mushrooms are polysaccharides and triterpenes. Eight triterpene compounds (**10** – **17**) have been isolated from *Antrodia camphorata*, comprising five lanostane (**11** – **13**, **15**, **17**) and three ergostane type compounds (**10**, **14**, **16**). The structures of these eight isolates are shown below in Figure 1.11.



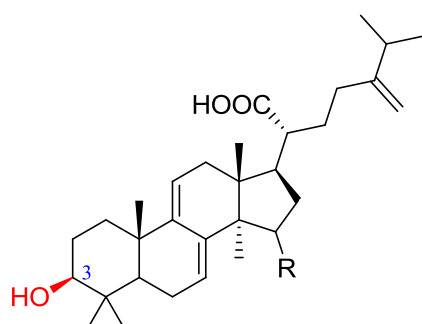
10: $R^1 = (=O)$, $R^2 = H$, $R^3 = CH_3$

14: $R^1 = (=O)$, $R^2 = R^3 = H$

16: $R^1 = R^2 = \alpha-OH$, $R^3 = H$



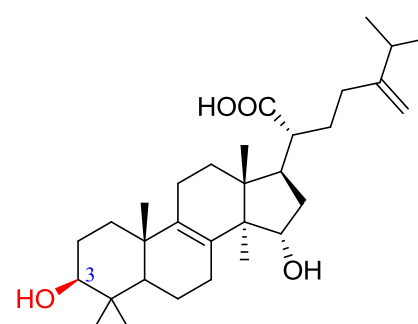
13



11: $R = H$

12: $R = \alpha-OAc$

15: $R = \alpha-OH$



17

Figure 1.11

Several studies have been undertaken on these compounds in order to establish their anti-tumour properties against various cancer cell types.^{49,56,57} The most potent isolates were found to be **10**, **14**, **16** as can be seen from Table 1.1 below. These compounds displayed a strong cytotoxic effect with IC_{50} values of 22.3 -75.0 μM against ten cancer cell types.

Table 1.1 IC₅₀ values (μM) of compounds **10** - **17**

Cell type	Cell Line	IC ₅₀ values (μM) of compounds 10 - 17							
		10	11	12	13	14	15	16	17
Colon	HT-29	22.3	387	59.8	233	30	inact	28.0	293
	HCT116	32.1	336	79.9	274	38.3	inact	42.6	289
	SW480	27.3	inact	86.5	276	30.7	inact	44.0	254
Liver	Huh7	30.4	inact	134	inact	42.8	inact	45.1	428
	HepG2	23.4	inact	inact	inact	38.6	inact	48.7	171
	Hep3B	68.3	inact	326	inact	48.4	inact	50.7	476
Breast	MDAMB231	25.1	370	55.9	133	25.6	399	27.8	89.2
	MCF-7	57.8	inact	inact	376	36.1	inact	53	357
Lung	A549	25.1	129	inact	312	41.9	194	34.4	inact
	CL1-0	31.5	140	inact	476	75	241	64.3	inact

Inact = IC₅₀ value > 500 μM

Zhankuic acid **14** was found to be the most inhibitory of the eight isolates on average over the 10 cancer cell lines screened. It has several defining chemical features including ketone moieties at positions 7 and 11 on the steroid ring, monomethylation at position 4, and alkene and carboxylic acid functionality at positions 24 and 25 respectively. The cytotoxicity of **14** can be seen clearly from Figure 1.12, which depicts the IC₅₀ values of **14** across ten cancer cell lines.

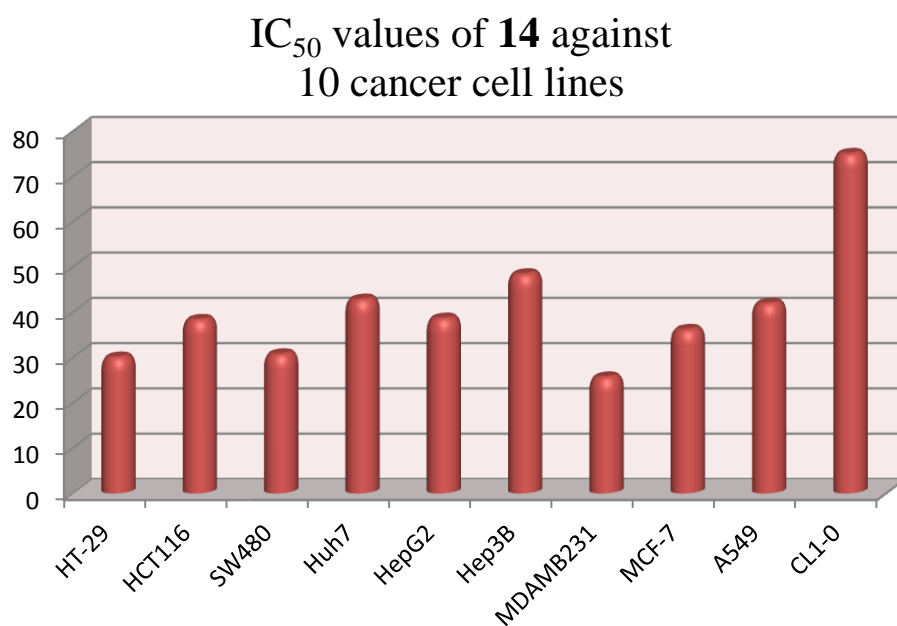


Figure 1.12

The wood-decay fungus *Wolfiporia extensa* is also used in traditional Chinese remedies. In combination with other herbs, it shows diuretic, sedative and analgesic effects.⁵⁸ Lanostane type triterpene acids isolated from the dried sclerotia of *W. extensa* have displayed anti-tumour, anti-inflammatory and anti-oxidant activities.⁵⁹⁻⁶⁵ She *et al.* have recently isolated three new lanostane steroids (**18** – **20**) which contain the interesting conjugated diene motif across the B and C rings.⁶⁶ The structures of the new isolates are shown in Figure 1.13 below, their biological activities remains to be assessed.

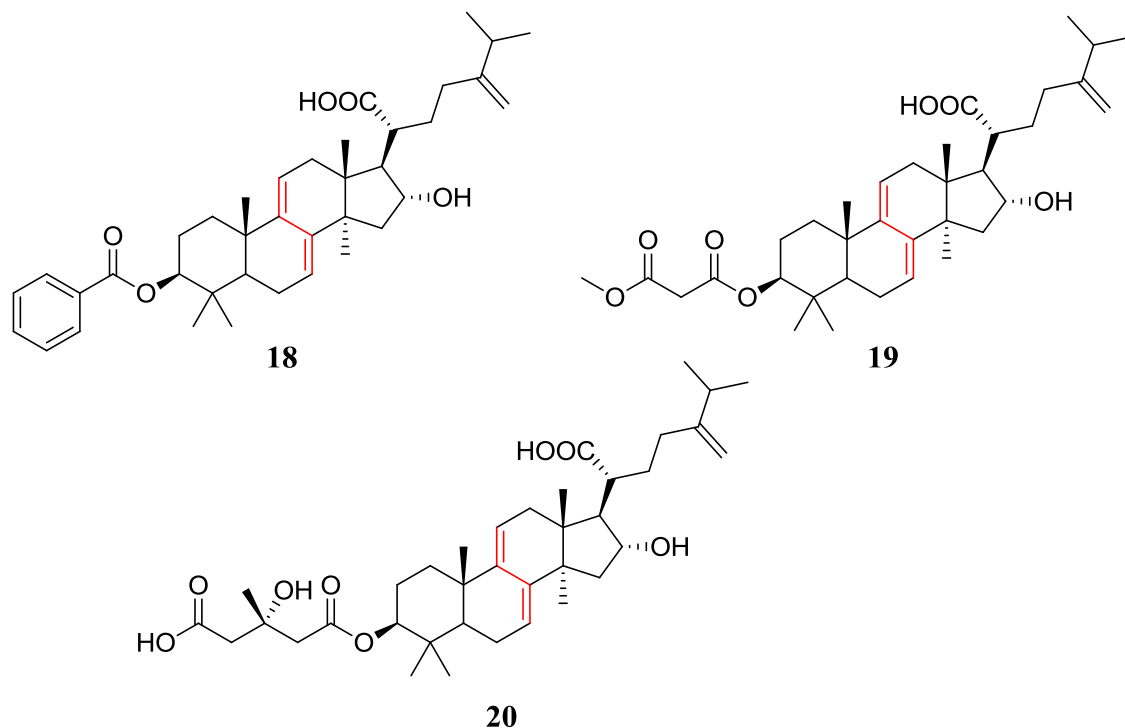


Figure 1.13

The anticancer activities of four new lanostane acids, isolated from the edible mushroom *Astraeus odoratus*, have been described by Arpha *et al.*⁶⁷ The mushroom is widely known for its popularity in Thai food dishes and is one of the most expensive edible mushrooms available.⁶⁸ Astraodoric acids A-D (**21** – **24**) isolated from the mushroom, shown below in Figure 1.14, display high levels of oxidation in the lanostane side chain. They were evaluated against three cancer cell lines, namely human carcinoma in the mouth, human lung cancer and human breast cancer.

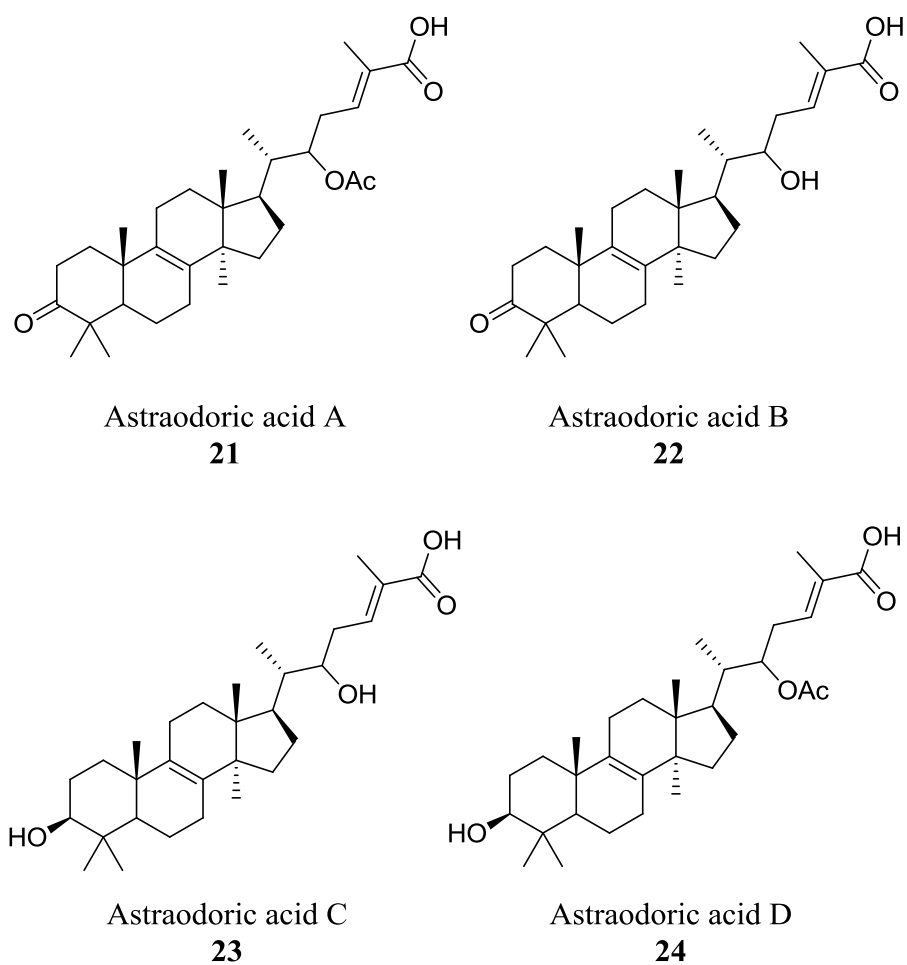


Figure 1.14

Table 1.2 Cytotoxicity of Astraodoric acids

	IC ₅₀ µg/mL		
	KB ^a	NCI-H187 ^b	MCF-7 ^c
Astraodoric Acid A	34.69	18.57	Inactive ^d
Astraodoric Acid B	19.99	48.37	Inactive ^d
Astraodoric Acid C	Inactive ^d	Inactive ^d	Inactive ^d
Astraodoric Acid D	31.55	34.15	40.15

^a Human mouth carcinoma, ^b human lung cancer, ^c human breast cancer, ^d Inactive at > 50 µg/mL

Cytotoxic activity against KB and NCI-H187 cell lines was displayed by Astraodoric acids A **21** and B **22**, while Astraodoric acid C **23** was inactive against all three cancer cell lines at concentrations above 50 $\mu\text{g/mL}$. Astraodoric acid D **24** exhibited slight cytotoxicity against all three cell lines. The best biological result in this study was the IC_{50} value of 18.57 $\mu\text{g/mL}$ seen for Astraodoric acid A in the NCI-H187 cell line. It is worth noting that the active compound contains a ketone moiety at C-3, which became important in this research.

New lanostane steroids (**25** – **28**) have also recently been isolated from the Korean wild mushroom *Naematoloma fasciculare*.⁶⁹ The bitter poisonous mushroom *N. fasciculare* grows on the stumps of old trees.⁷⁰ The mushroom is well known for its diverse phytochemical constituents including ergosterols, triterpenoids and sesquiterpenoids,⁷¹⁻⁷⁷ and particularly for the presence of lanostane triterpenoids as the toxic components.⁷²⁻⁷⁶ Kim *et al.* isolated four new bioactive molecules from the mushroom *N. fasciculare*, which are shown below in Figure 1.15.

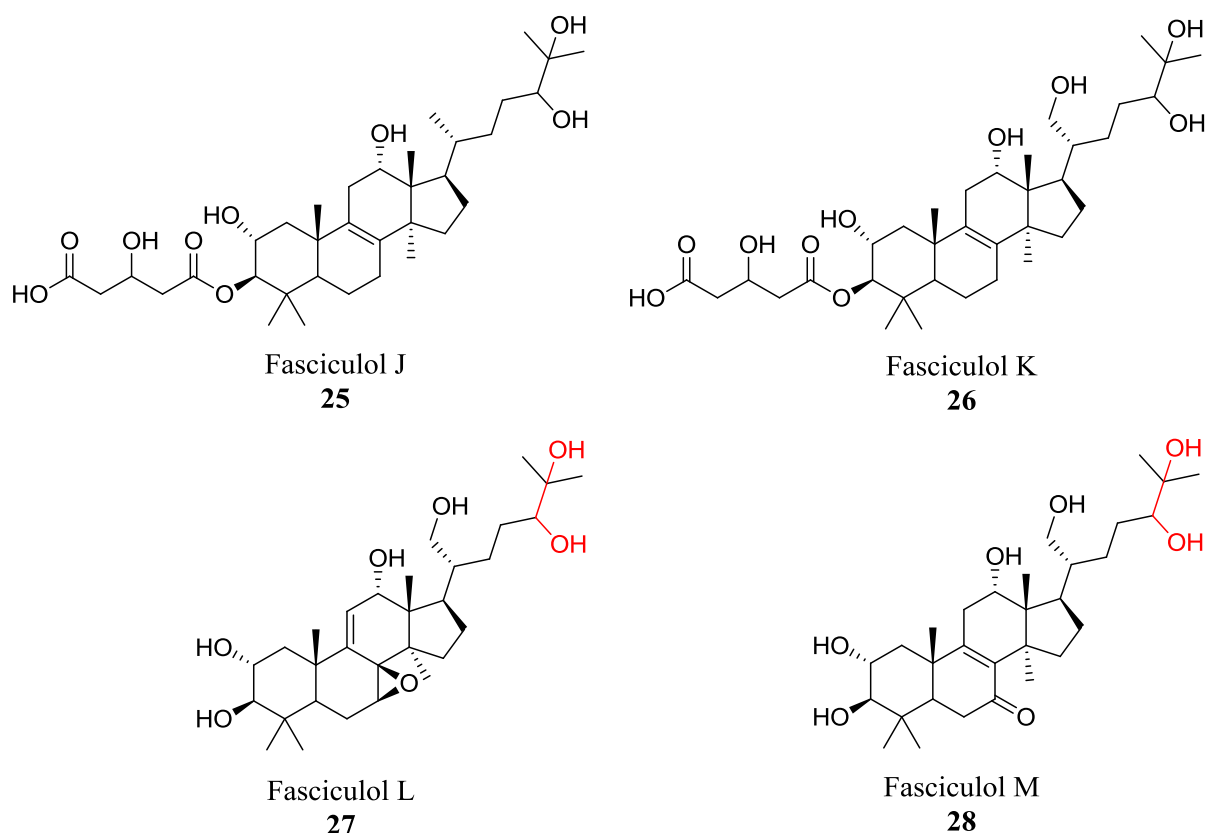


Figure 1.15

The isolates were evaluated for their cytotoxicity against four human cancer cell lines, namely A549 (non-small-cell lung adenocarcinoma), SK-OV3 (ovary malignant ascites), SK-MEL-2 (skin melanoma) and HTC-15 (colon adenocarcinoma), and the results are presented below in Table 1.3.

Table 1.3

	IC ₅₀ (μM)			
	A549	SK-OV-3	SK-MEL-2	HCT-15
Fasciculol J 25	>30	>30	>30	>30
Fasciculol K 26	>30	>30	>30	>30
Fasciculol L 27	6.59	7.08	8.26	8.53
Fasciculol M 28	3.99	7.36	4.77	8.50

Kim *et al.* state that Fasciculols J and K (**25** and **26**) showed no cytotoxic activity against the four human cancer cell lines, however Fasciculols L and M (**27** and **28**) showed moderate cytotoxicity.⁷¹ The positive control used in this study was the compound doxorubicin which has IC₅₀ values of 0.02, 0.01, 0.01 and 0.13 μM against A549, SK-OV3, SK-MEL-2 and HTC-15 respectively. A notable structural feature of the active lanostanes is the diol moiety at C-24,25 in the side chain. This functionality will be incorporated into the side chain through the course of this research. The main difference between the inactive compounds Fasciculols J **25** and K **26**, and the active Fasciculols L **27** and M **28**, is the long acid side chain at C-3. The active compounds have less sterically bulky alcohols at this position, which may improve biological activity due to hydrogen bond forming potential.

In addition to lanostane derivatives being present in fungi, they have also been isolated from plant sources. Four interesting lanostane type compounds were isolated by Lu *et al.* from the plant *Euphorbia humifusa*, which is found in eastern Asia and central Europe.⁷⁸ *Euphorbia humifusa* has been used in traditional Chinese medicine for the treatment of dysentery, enteritis and hematochezia.⁷⁹

Figure 1.16 *Euphorbia humifusa*

The four lanostanes isolated (**29** – **32** Figure 1.17) are relevant to this research because they possess oxygen functionalities at key sites in the lanostane structure, namely C-7 and C-11. Ketones in these positions give rise to the conjugated diketone moiety also seen in isolates from *Antrodia camphorata*.

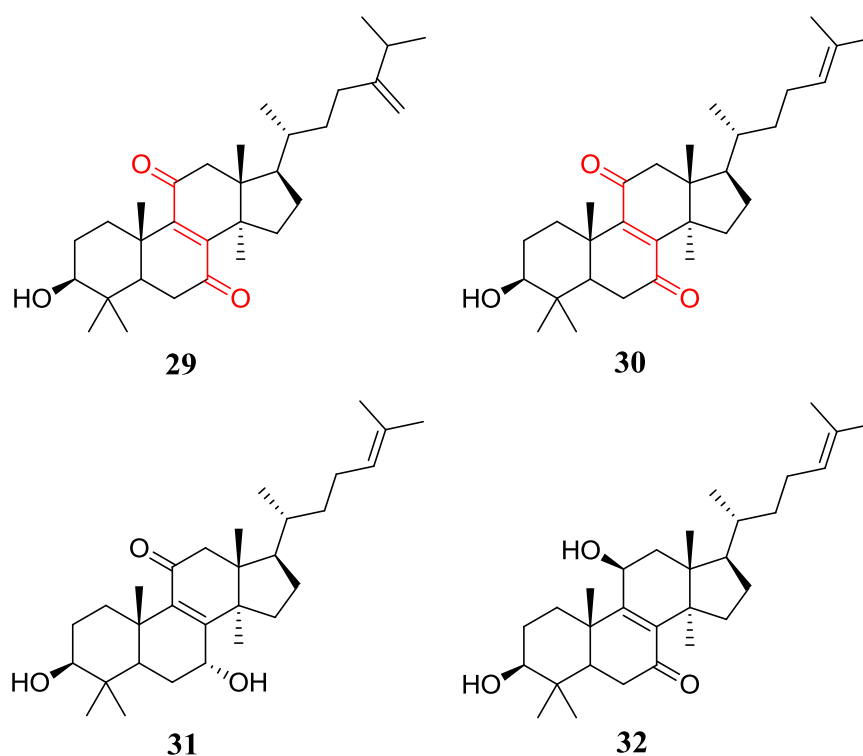


Figure 1.17

Though these compounds were not biologically evaluated in the study by Lu, their isolation shows the relevance of lanosterol oxidation products in natural sources.

Another natural source of lanostanes is the white rot fungus *Inonotus obliquus*, which is widely distributed across Europe and North America, and also found in Africa and Asia (Figure 1.8).⁸⁰ The relatively diverse locations of *I. obliquus*, as compared to *A. camphroata* which is only found in the mountains of Taiwan, give rise to a high level of interest in its isolates from the scientific community. Thus, several studies on *I. obliquus* have been reported.⁸¹⁻⁸⁷



Figure 1.18 Worldwide locations of the white rot fungus *Inonotus obliquus*

Nakamura *et al.* report the isolation of six new lanostane type triterpenoids (**33** – **38**) from the methanolic extract of the sclerotia of *I. obliquus* (Figure 1.9 below). This methanolic extract was shown to have an inhibitory effect on HT1080 cells.⁸⁰ The triols (**33** and **34**) highlighted in Figure 1.19 contain interesting structural features in terms of work within our research, as discussed in Chapter 2.

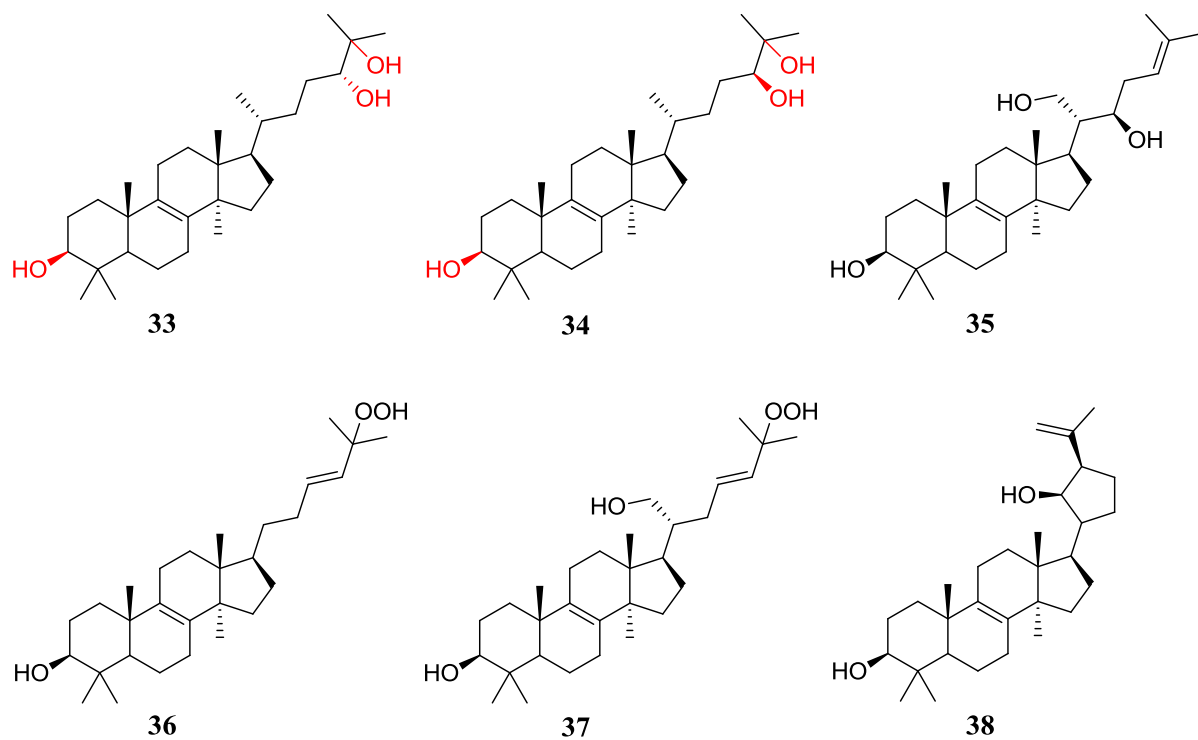


Figure 1.19

1.9 Chemical Reactivity of Lanosterol

The synthetic chemistry of lanosterol revolves around the functionality intrinsic in the compound. The C-8,9 alkene is ideal for exploitation *via* allylic oxidations or epoxidations. Allylic oxidation of the tetrasubstituted alkene affords ketones at positions 7 and 11 in the steroidal ring system, comparable to isolates **10**, **14** and **16** from *A. camphorata* (Figure 1.11). The side chain C-24,25 alkene provides a platform for a variety of oxidations, including the possibility to oxidatively cleave the side chain and thus explore synthetic chemistry beyond the lanostane nucleus. Additionally, the C-3 alcohol is an excellent target for oxidations, which can be carried out under much milder conditions than the harsh allylic oxidations. Literature precedent for steroidal ring and side chain modifications are discussed below in further detail.

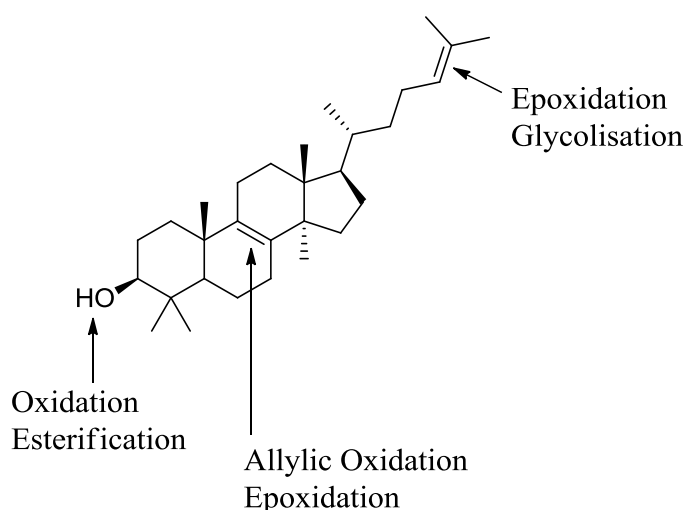
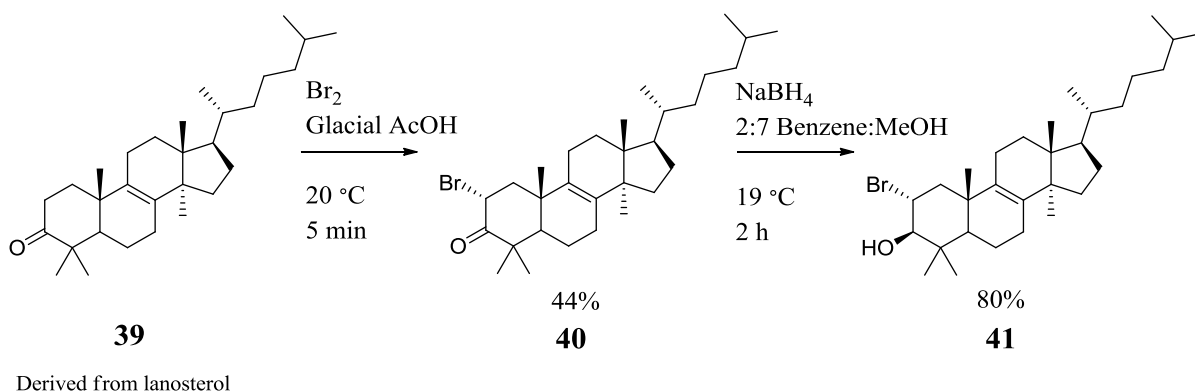


Figure 1.20 Sites of Chemical Reactivity in Lanosterol **1**

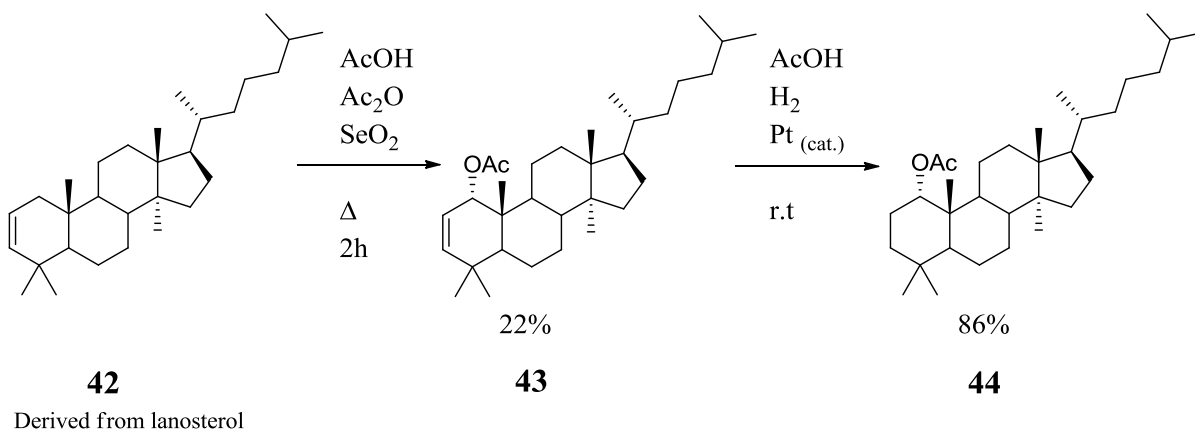
1.10 Synthetic Lanosterol Derivatives and their Biological Activities

Early work in the area of synthesising lanostane derivatives was carried out by McGhie and Barton.⁸⁸⁻⁹² Initial reactions in 1957 involved transformations on the A ring of the steroid *via* brominations and reductions (Scheme 1.2).⁸⁸



Scheme 1.2

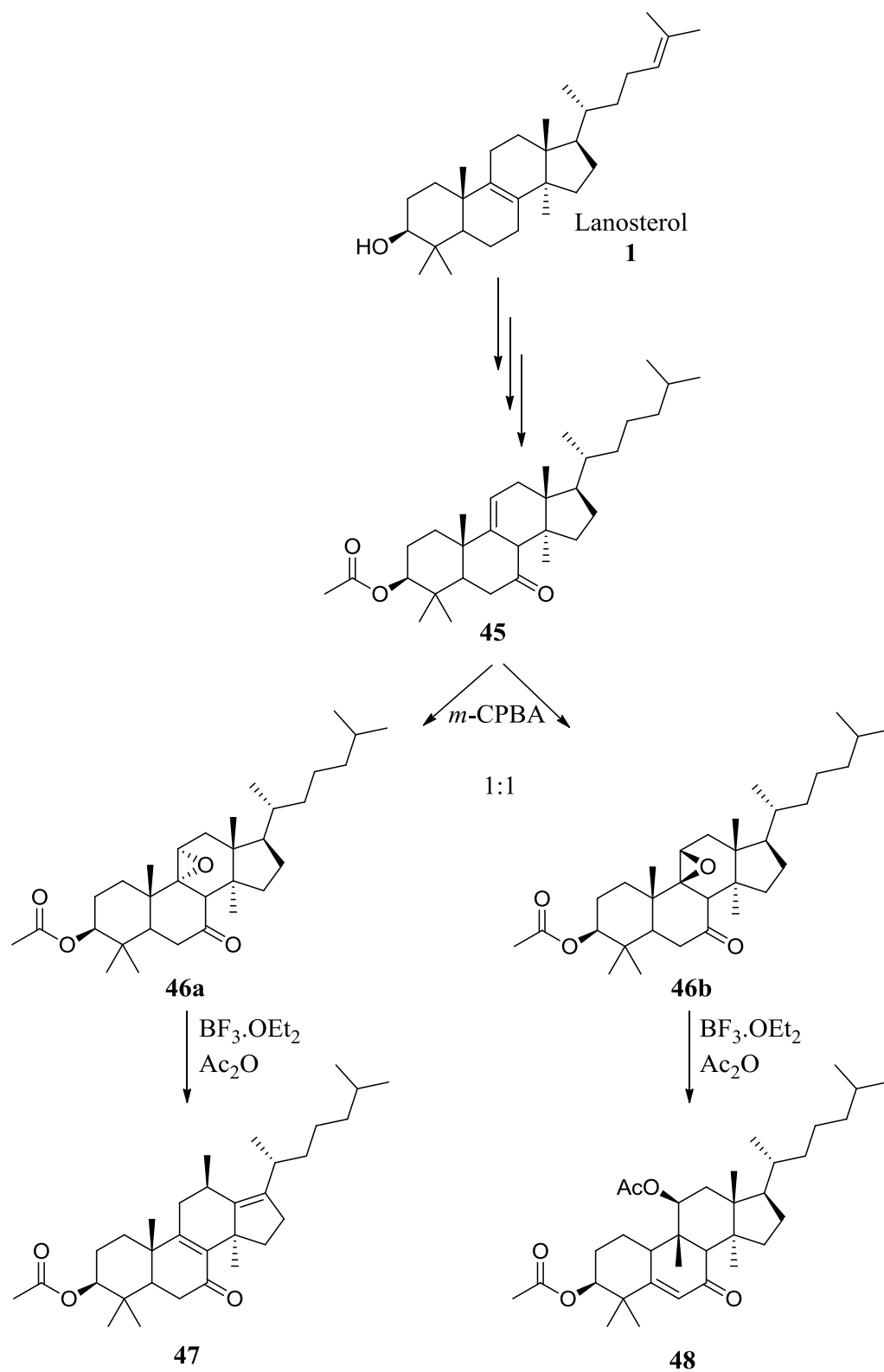
This work was elaborated on by McGhie and Barton in 1963 by further derivatising the A ring *via* acetylation and reduction (Scheme 1.3).⁹⁰



Scheme 1.3

More recently, Parvez *et al.* describe the manipulation of the lanostane B and C rings in experiments directed at transforming the lanostane skeleton into that of curcubitane, which possesses a methyl group at C-10 and the alkene bond is repositioned to C-5,6.⁹³

Towards this end, two epoxides were prepared by the *m*-CPBA oxidation of 3 β -acetoxy-lanosta-9(11)-en-7-one **45**,^{94,95} one epoxide being formed on the α face **46a**, the other on the β face **46b**. Upon treatment of these epoxides with boron trifluoride, the α epoxide yielded the lanostane derivative 3 β -acetoxy-18(13 \rightarrow 12 β)-*abeo*-lanosta-8,13(17)-dien-7-one **47**, while the β epoxide yielded the curcubitane derivative 3 β ,11 β -diacetoxy-10 α -curcubit-1(10)-en-7-one **48** (Scheme 1.4).



Scheme 1.4

Note: mass yields for unpurified materials only are described by Paryzek.⁹³
The products shown are described as the “major” products of these pathways.

The variation of these final structures, formed by α and β forms of the same compound **46**, shows how diverse products can be synthesised from the lanostane nucleus.

Paryzek *et al.*⁹³ describe the ring opening of the α and β lanostane epoxides **46** and the subsequent cascade which yields a cucurbitacin skeleton. Following ring opening of the epoxide, carbocationic intermediates are formed, which invoke a series of 1,2 methyl and hydride shifts. It has been established that the carbon oxygen epoxide bond at C-9 must break prior to methyl migration, and that two factors contribute to the direction of the migration of the methyl group from its position at C-10 to C-9. Firstly, the steric interaction between the migrating methyl group and the bulky Lewis acid/acetic anhydride complex facilitates the $10\beta \rightarrow 9\beta$ methyl shift. An additional directive effect is exerted by the 1,3-diaxial repulsion between the C-4 methyl group and the C-10 methyl group. Both of these factors combine to transform the lanostane skeleton into the cucurbitane skeleton, as can be seen in Figure 1.21.

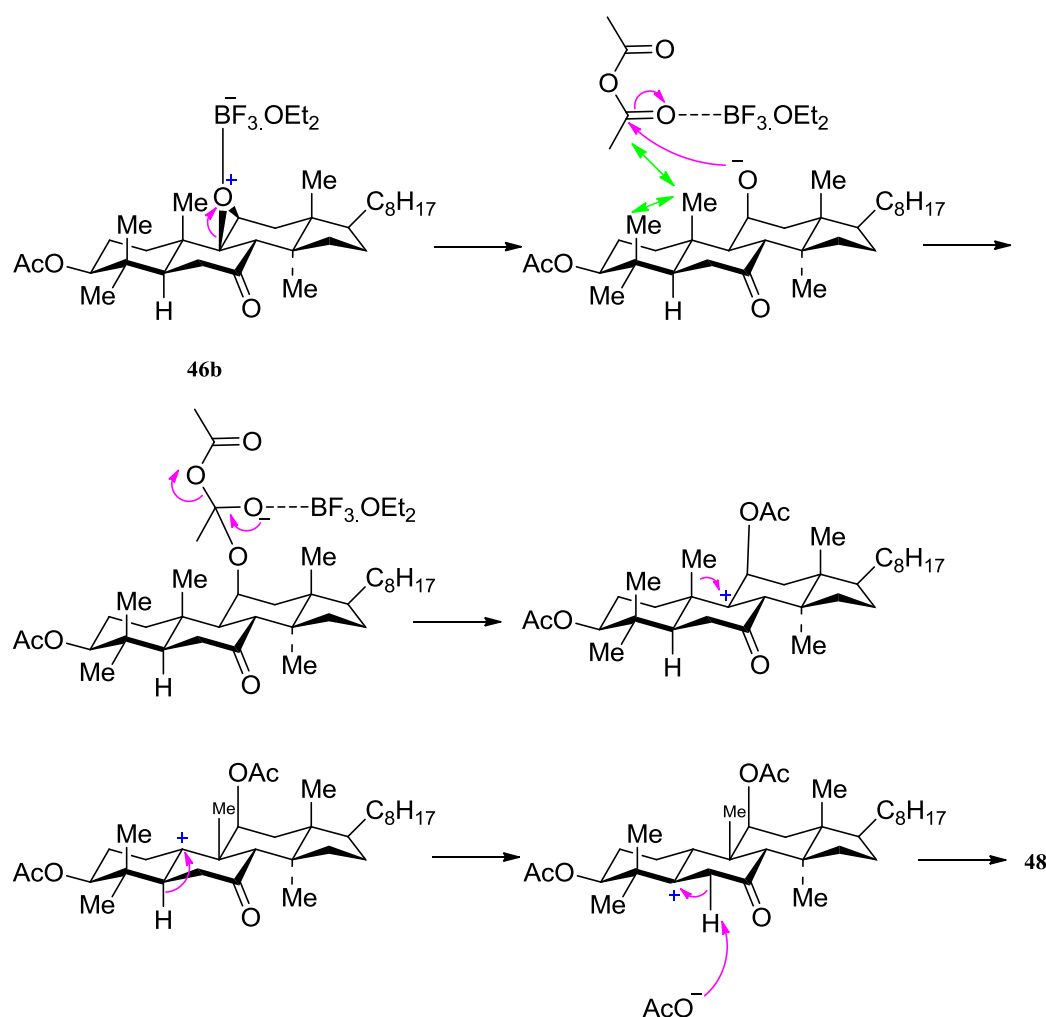
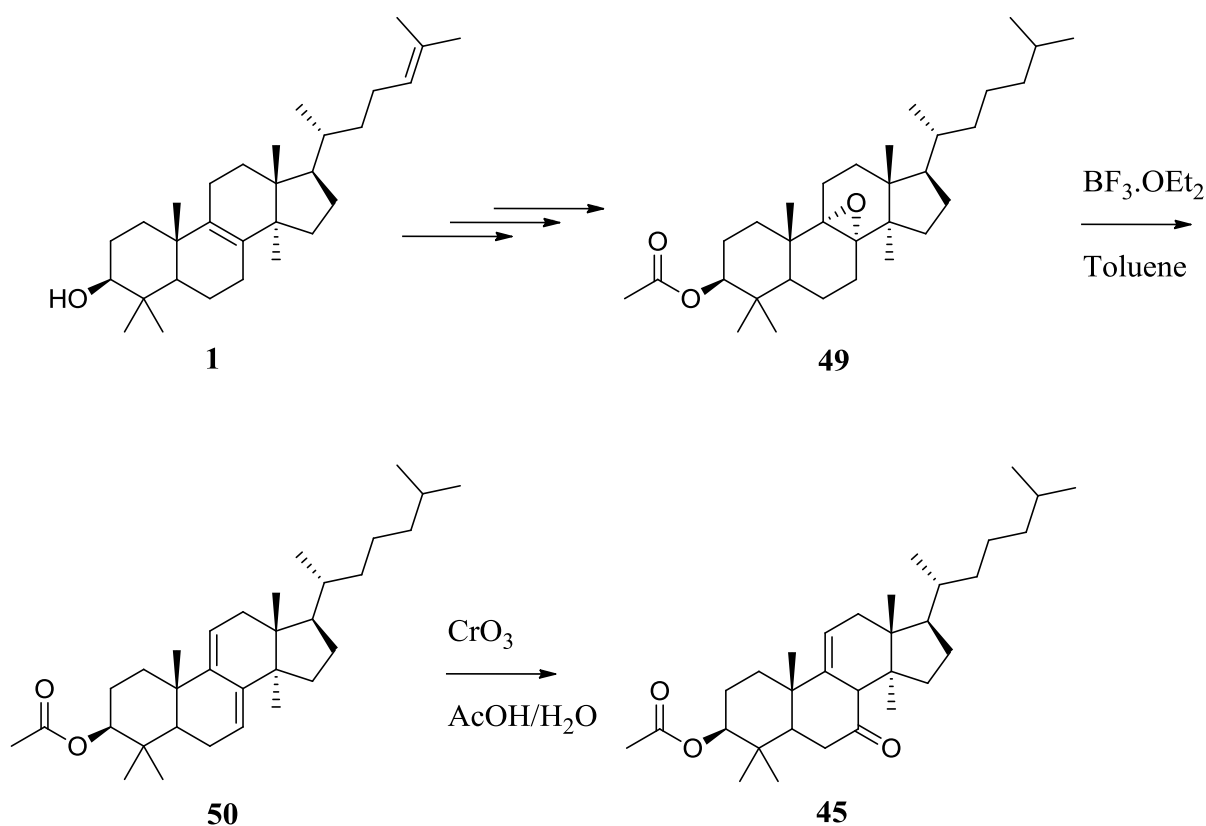


Figure 1.21

This rearrangement is stereoselective to an extent, in that the β epoxide undergoes ring opening and rearrangement to form the cucurbitane skeleton as the major product, while the α epoxide ring opens under the same conditions but the subsequent cascade has been shown to yield the lanostane derivative **47** as the major product.

This result shows the diversity of products available from the ring opening of epoxides in the steroidal rings.

Briggs showed that ring opening of the C-8,9 epoxide **49** using boron trifluoride leads to a different chemical outcome.⁹⁶ As can be seen from Scheme 1.5, in this instance, the diene at C-7,8 and C-9,11 is formed **50**. This diene can be subsequently oxidised to form 3 β -acetoxy-lanosta-9(11)-en-7-one **45**.



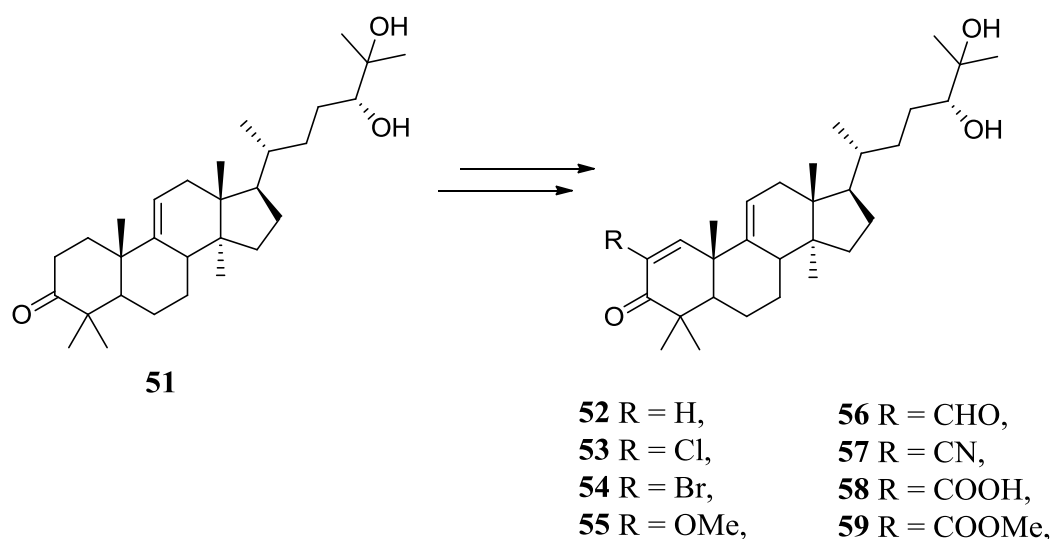
Note: Briggs does not report yields for these transformations

Scheme 1.5

It is clear from these examples of modification of the steroidal rings that the C-8,9 alkene has enormous potential for the synthesis of a diverse range of functional groups. While this alkene opens up a variety of chemistry for the steroidal rings, the alcohol at C-3 in the lanostane structure can also be exploited for functional group transformations and nearby carbon derivatisations.

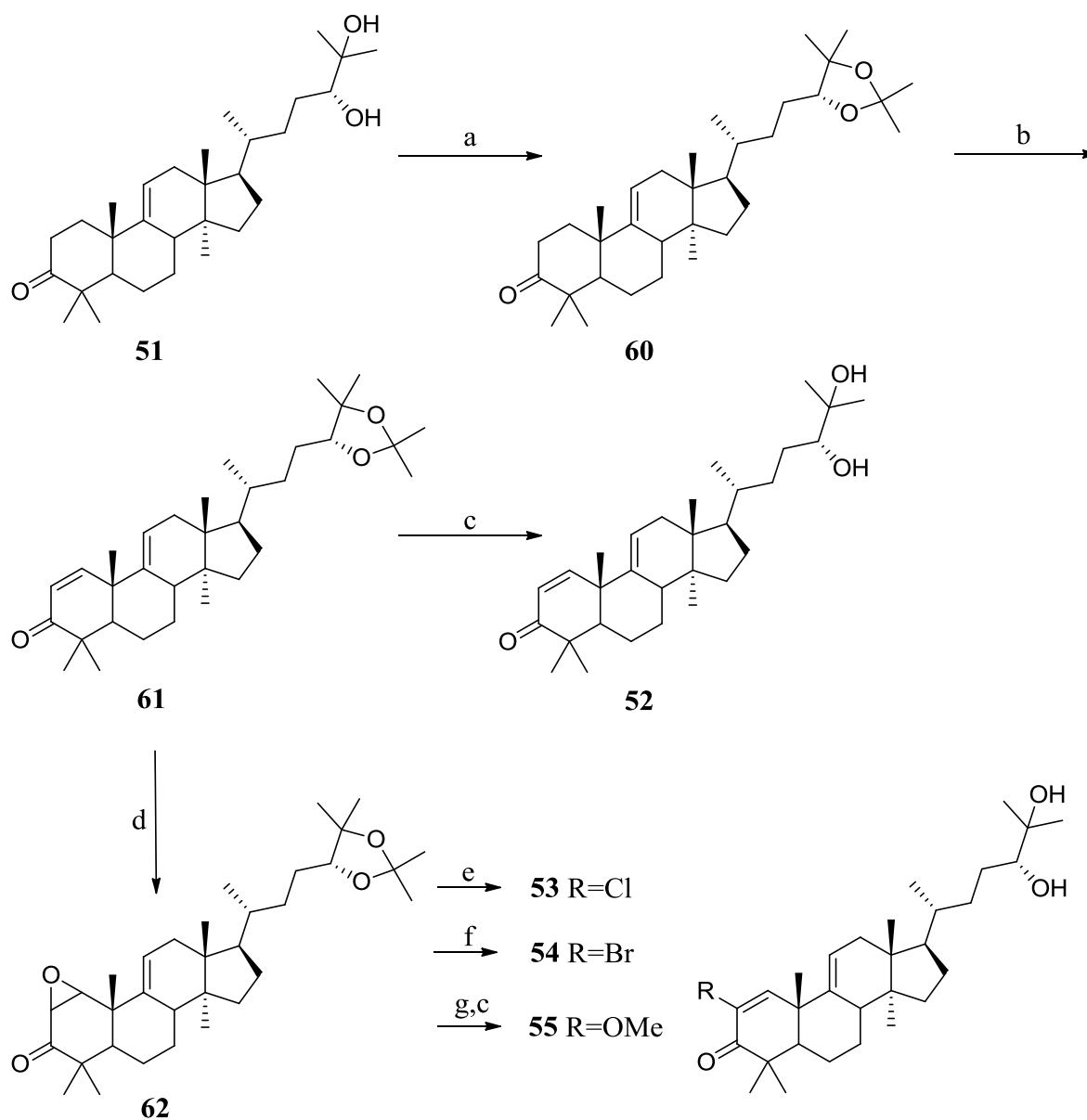
Given the biological activity of the lanostane type isolates from natural sources, along with the accessibility of a wide range of derivatives, it is unsurprising that much research attention has been focussed on synthetic lanosterol derivatives and their biological activities.

Wada *et al.* have synthesised derivatives shown below from 3-oxolanost-9(11)-en-24,25-diol **51**, which was isolated from *Pinus luchuensis* (Scheme 1.6).⁹⁷



Scheme 1.6

Compounds **52** - **55** were synthesised *via* a similar synthetic sequence. Firstly, the protection of the C-24,25 diol **51** by forming the acetonide **60** was achieved with dimethoxypropane. Phenylselenenyl chloride followed by hydrogen peroxide facilitated the formation of a double bond at C-1 **61**, while C-2 was then derivatised, *via* intermediate **62**, using hydrochloric acid, hydrobromic acid and sodium methoxide to yield **53**, **54** and **55** (Scheme 1.7).



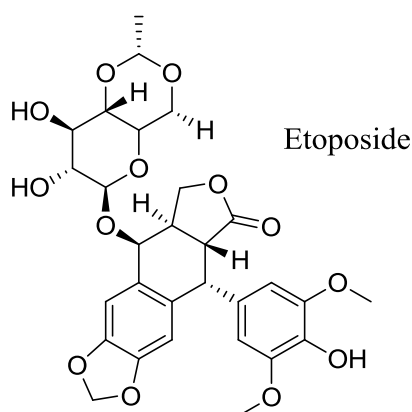
a = dimethoxypropane, TsOH, DCM, (90%); b = PhSeCl, EtOAc, then H₂O₂, THF, (78%); c = 1N HCl, DCM, MeOH, (89%); d = H₂O₂, NaOH, THF, (65%); e = HCl, AcOH, CHCl₃, (60%); f = HBr, AcOH, CHCl₃, (63%); g = NaOMe, MeOH, (54%).

Scheme 1.7

Compounds **56** – **59** were synthesised *via* another synthetic route, but a similar initial protection of the diol was carried out as per the previous sequence.⁹⁷

The biological activity of these synthetic derivatives was evaluated against DNA topoisomerase II, one of the target enzymes for chemotherapeutic drug development. The cytotoxic activity of the compounds against A549 cells was also examined. The results are displayed in Table 1.4.

Table 1.4



Entry	Compound	IC ₅₀ [*] (μM)	ED ₅₀ [*] (μM)
1	52	149.97 ± 2.33	26.90 ± 4.58
2	53	106.80 ± 5.98	38.15 ± 5.18
3	54	18.27 ± 2.20	14.27 ± 4.81
4	55	22.77 ± 6.06	29.57 ± 1.60
5	56	1.86 ± 2.57	13.05 ± 2.59
6	57	83.23 ± 5.65	25.49 ± 3.54
7	58	23.80 ± 3.35	7.33 ± 1.31
8	59	66.51 ± 5.51	3.96 ± 0.31
9	Etoposide	50.40 ± 3.29	3.39 ± 0.61

* IC₅₀ values against topoisomerase II activity and ED₅₀ values against A549 cells

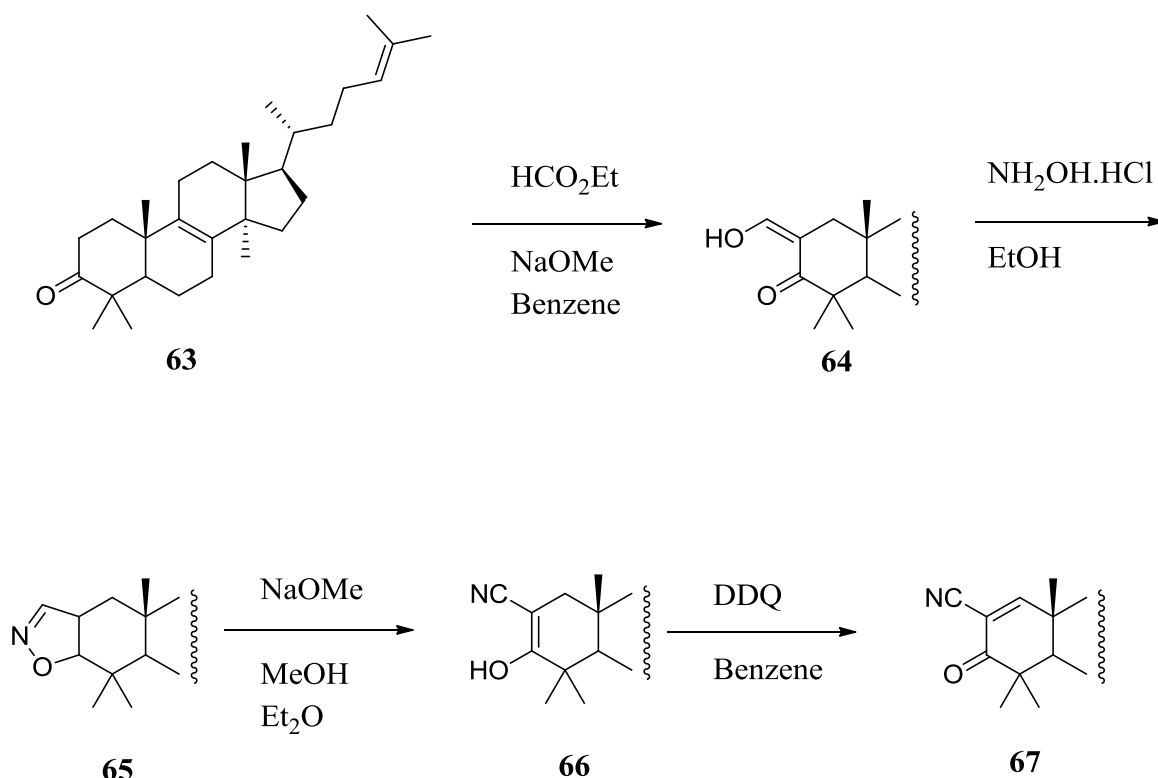
All the compounds inhibited Topoisomerase II activity with IC₅₀ values ranging from 1.86 to 149.97 μM. Compound **56** showed the highest inhibitory effect, having an IC₅₀ value of 1.86 μM. Perhaps the ability of the aldehyde to exhibit an electrostatic effect with the enzyme gave rise to this high inhibitory effect.

In terms of cytotoxic activity, acid **58** and ester **59** exhibited potent activity with ED₅₀ values of ~ 7 and ~ 4 μM respectively, in contrast to their Topoisomerase II activity. Particularly

excellent results were displayed by the methyl ester derivative **59** in terms of a comparison with etoposide, as can be seen from Table 1.4.

These results show that the introduction of functional groups at C-2 of the triterpenoid skeleton have increased the Topoisomerase II inhibitory effect and the cytotoxic activity.

Honda *et al.* also report C-2 derivatised lanostane products, and discuss an interesting synthesis of a novel cyanoenone derivative from lanost-8-en-3-one **63** (Scheme 1.8).⁹⁸

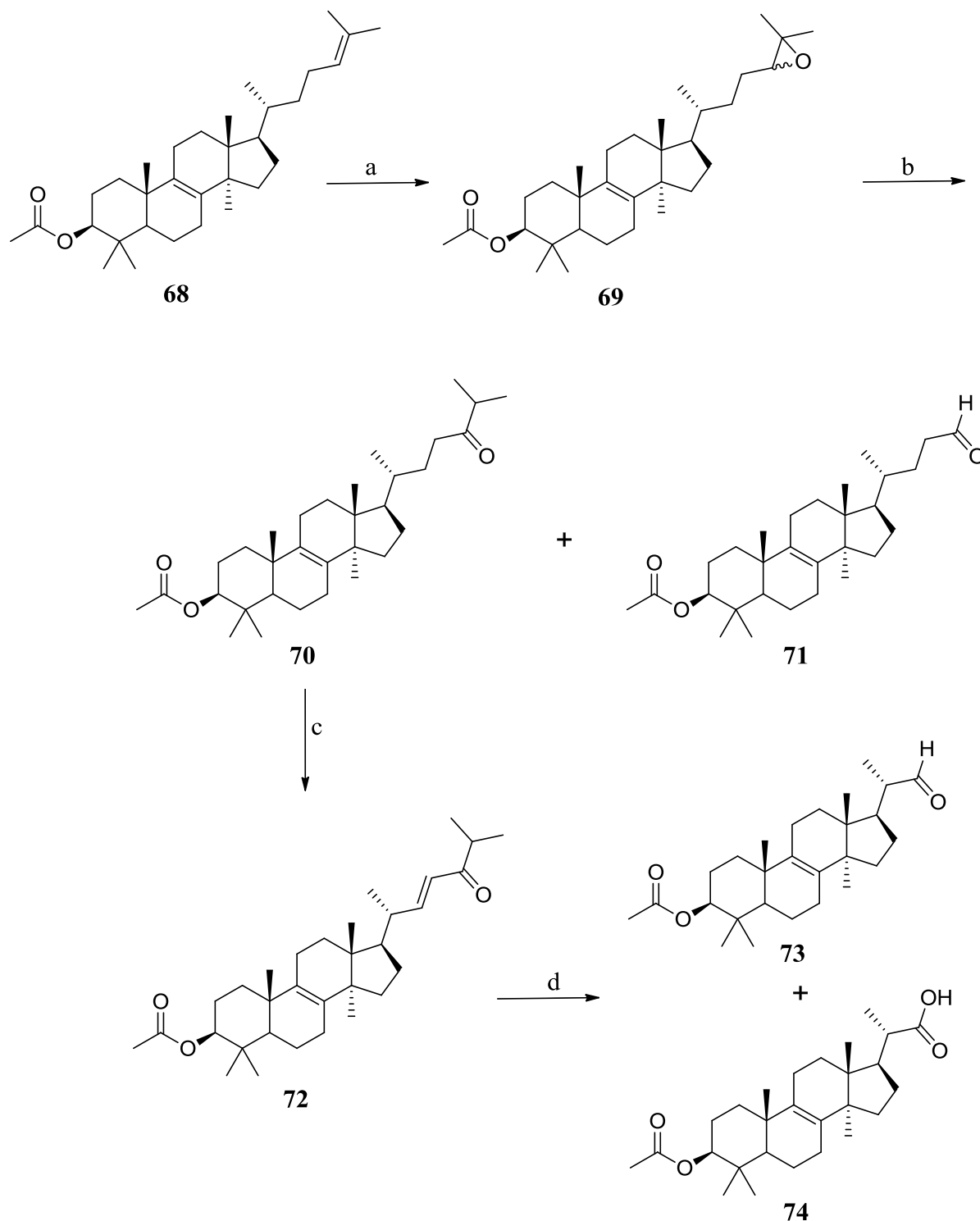


Scheme 1.8

Formylation of the starting material lanost-8-en-3-one **63** gave the intermediate enol **64**, which was carried forward to yield the isoxazole **65**. Cleavage of the isoxazole with sodium methoxide gave the nitrile **66** which was subsequently treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to yield the desired cyanoenone **67**. Studies on the biological properties of this compound are in progress.

From these examples, it is evident that the steroid rings of lanosterol can be modified to include a wide range of functionalities by exploiting the C-8,9 alkene and the C-3 alcohol in the structure. There is also scope for further derivitisation of lanosterol in the side chain, due to the presence of the C-24,25 alkene.

Sato *et al.* describe the synthesis of lanosterol analogues with modified side chains starting from lanosterol acetate **68**. Functional groups including epoxides **69**, ketones **70** and **72**, aldehydes **71** and **73**, and acids **74** can be synthesised from the C-24,25 alkene, Scheme 1.9.⁹⁹

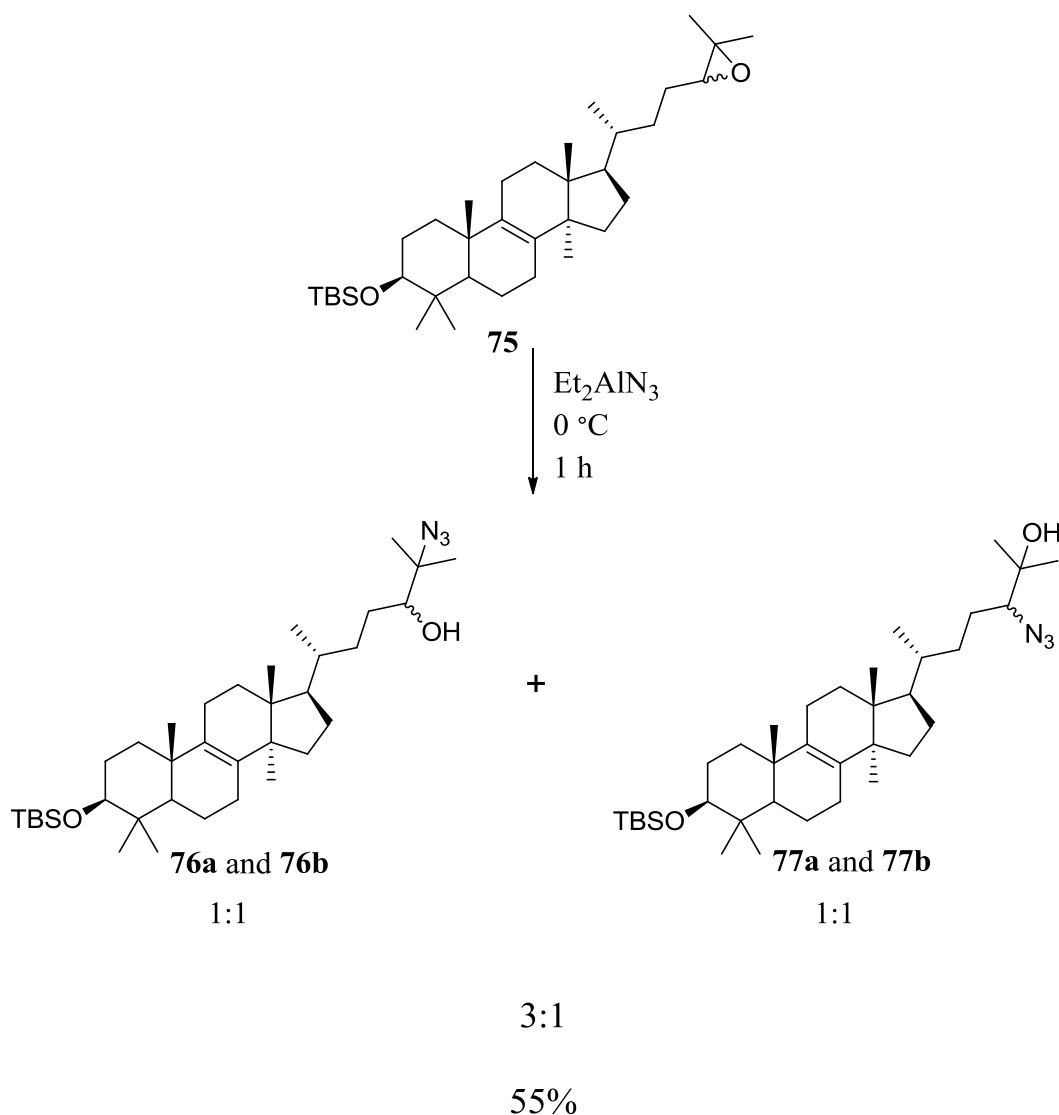


a = *m*-CPBA, CHCl_3 ; b = $\text{BF}_3 \cdot \text{OEt}_2$, benzene; c = SeO_2 , EtOH; d = KMnO_4 , acetone.
Sato *et al.* did not report yields for purified products.

Scheme 1.9

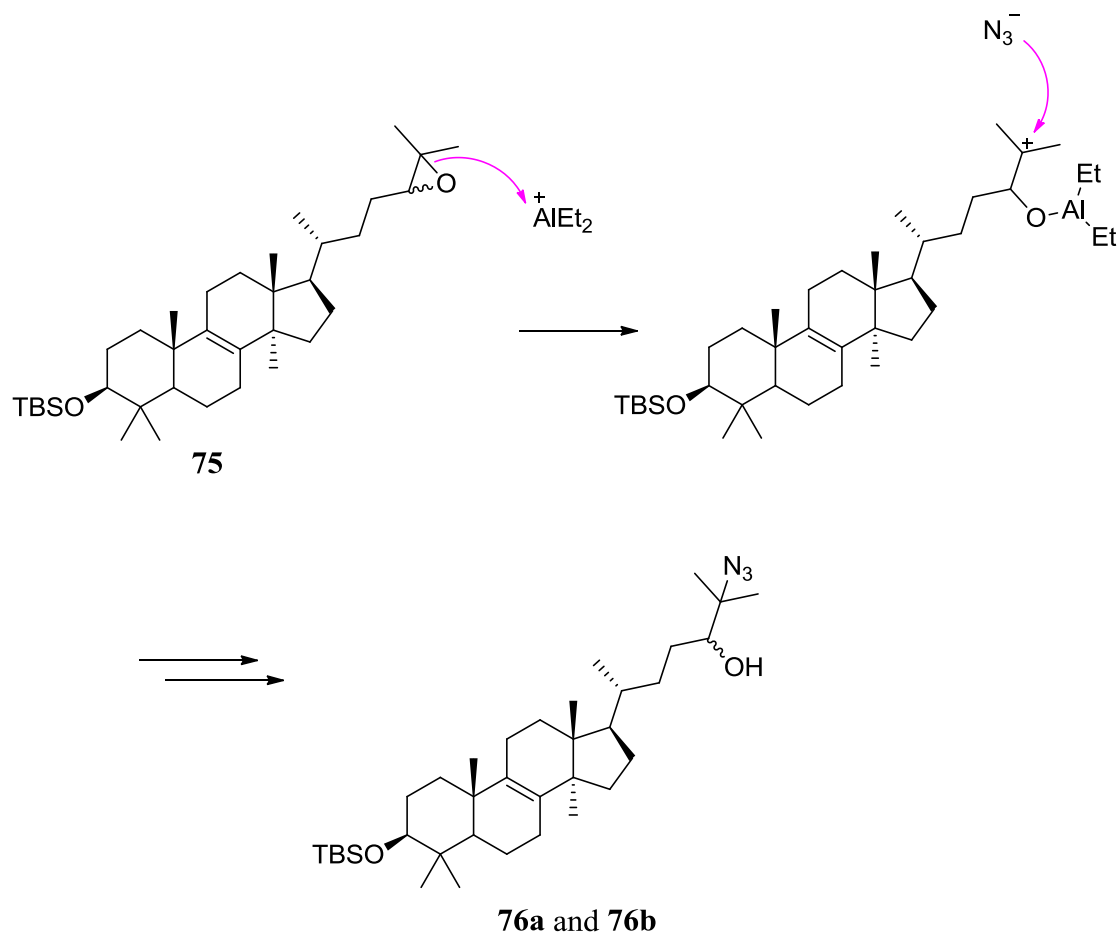
The C-24,25 alkene is the starting point for this chemistry and is the basis for the modification of the side chain to include epoxides, aldehydes, ketones and carboxylic acids.

The C-24,25 epoxide shown in Scheme 1.10 is a particularly useful intermediate, and can be derivatised beyond ketone and aldehyde functional groups, as shown by Davis *et al.*,¹⁰⁰ who report the formation of azohydrins (**76** and **77**) in the lanostane side chain. The diethylaluminium azide mediated opening of the 1:1 diastereomeric mixture of C-24,25 epoxides, protected at C-3 by tert-butyldimethylsilyl ethers, gives rise to a 3:1 mixture of tertiary and secondary azohydrins, both of which were 1:1 C-24 diastereomers, as shown in Scheme 1.10.



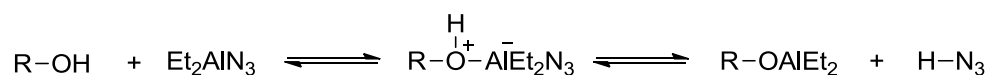
Scheme 1.10

The reaction of Et_2AlN_3 with the trisubstituted epoxide affords the tertiary azido alcohol **76** as the major product, consistent with an $\text{S}_{\text{N}}1$ -like mechanism. The major product arises from the ring opening of the epoxide and coordination of the oxygen to the aluminium, followed by attack of the azido species at the developing carbocationic centre (Scheme 1.11).



Scheme 1.11

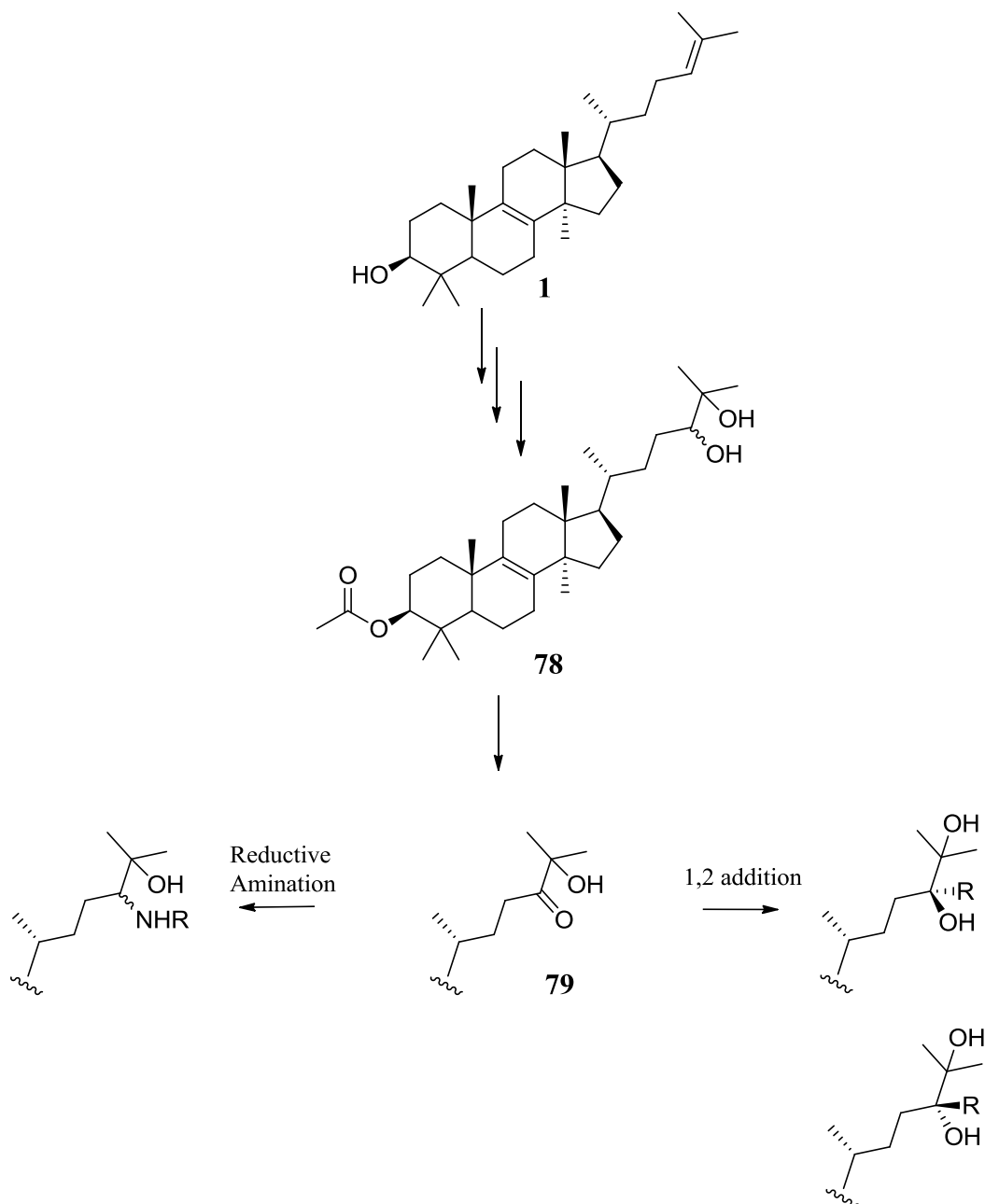
The C-3 protection of lanosterol is necessary in this case to avoid the possible formation of side products arising from hydrazoic acid generated by $\text{OH-Et}_2\text{Al}$ exchange (Scheme 1.2).



Scheme 1.12

The C-24,25 alkene can also be derivatised without the use of the epoxide intermediate, and an example of a useful moiety as a precursor to various functionalities is a 1,2 diol.

It is known that 1,2 diols may be converted to α -hydroxy ketones, and such transformations have been carried out by Corey and Kim.¹⁰¹ The side chain glycol, easily accessible from lanosterol, may thus be converted to the corresponding α -hydroxy ketone **79**. The synthetic versatility of these moieties has been highlighted by Plietker,¹⁰² who demonstrated the various functional groups which can be derived from α -hydroxy ketones (Scheme 1.13).



Scheme 1.13

In conclusion, the main sites of reactivity of lanosterol are the alcohol at C-3, the α carbon at C-2 and the alkenes at C-8,9 and C-24,25. By manipulation of these reactive sites, many transformations of the lanostane nucleus are possible as summarised below (Figure 1.22).

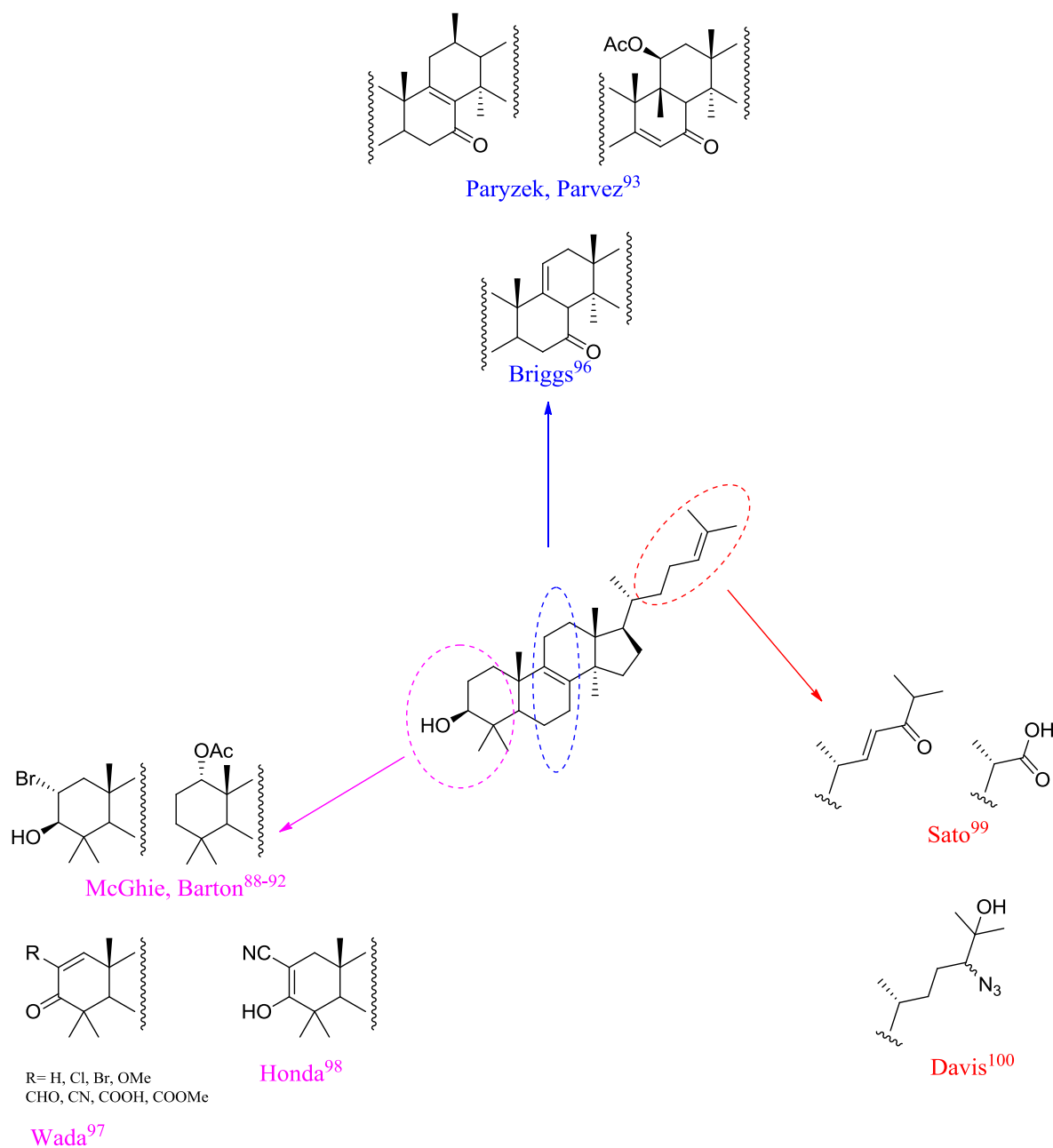


Figure 1.22

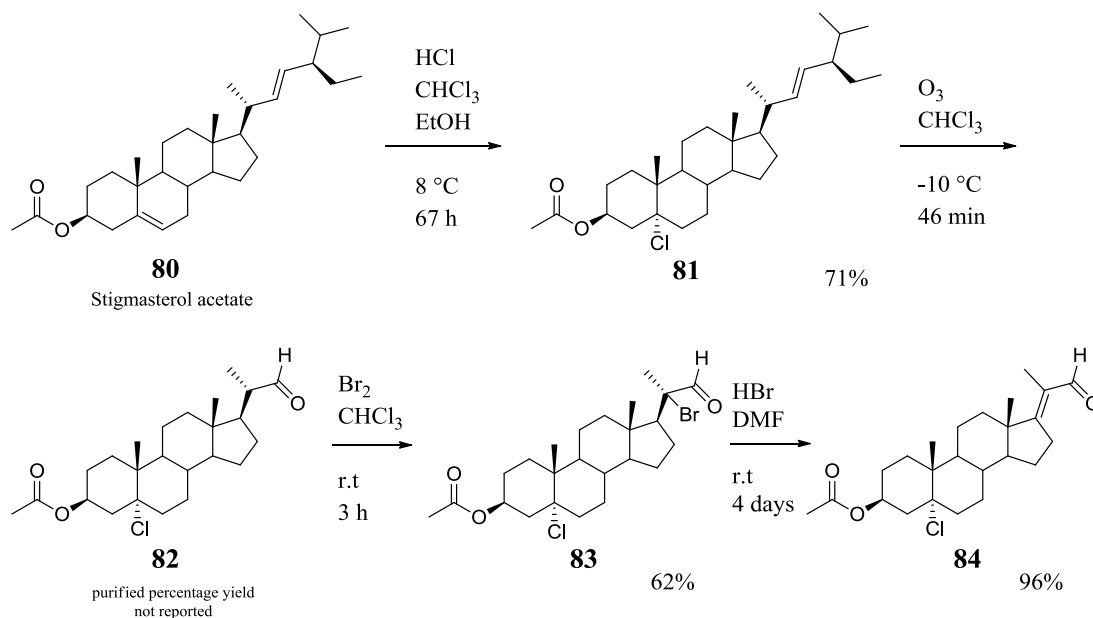
1.11 Synthetic Stigmasterol Derivatives and their Biological Activities

In the drive to reduce cholesterol absorption, an increase in dietary phytosterols has become prevalent in recent years, with esters of phytosterols being incorporated into an ever increasing number of food products, such as margarine and oils.¹⁰³⁻¹⁰⁸ In recent years, much work has been conducted on the negative health effects of cholesterol oxidation products (COP's), which include atherosclerosis, cytotoxicity and mutagenicity.¹⁰⁹⁻¹¹¹

The structural connection between cholesterol and the plant sterols infers that these compounds can undergo oxidation by similar means to those documented for cholesterol.^{16,112} Given the unsaturated nature of the phytosterols, there is a possibility of oxidation of the compound occurring upon exposure to air, a process which may be enhanced by heating, chemicals or enzymes.

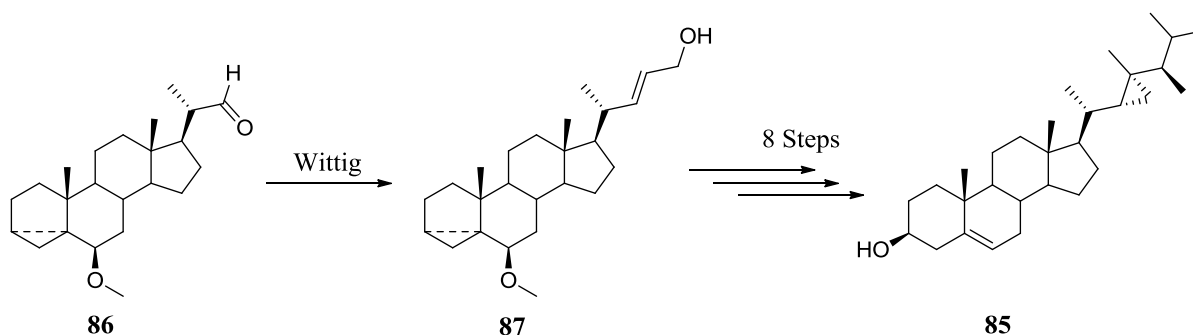
Therefore, due to the increase in dietary uptake of phytosterols, coupled with the knowledge that these compounds may undergo oxidation *in vivo* and the adverse effects of their cholesterol oxide analogues, the biological evaluation of the oxides of phytosterols is now an important research topic.

First and foremost, early work in this area was carried out in 1957 by Chamberlin *et al.* who performed a variety of synthetic transformations at multiple reactive sites in stigmasterol.¹¹³ This work involved halogenations at the C-5,6 alkene bond to yield **81**, and cleavage of the stigmasterol side chain *via* ozonolysis to yield **82**, as seen in Scheme 1.14.



Scheme 1.14

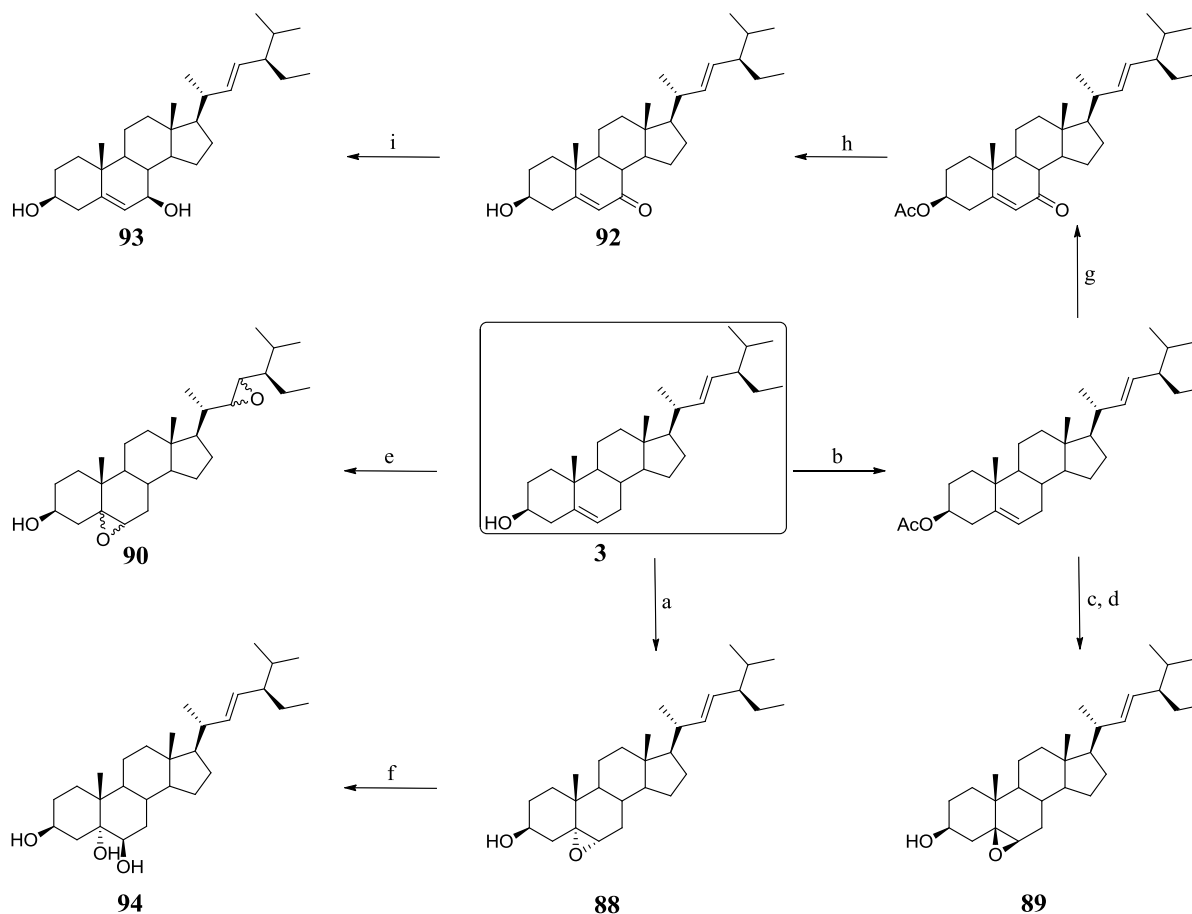
The formation of the C-22 aldehyde, as shown in Scheme 1.14 above was an attractive method for the derivitisation of the side chain of stigmasterol, and was also exploited by Terasawa *et al.* in 1983 en route to the stereocontrolled synthesis of gorgosterol **85** in 9 steps *via* **86** and **87** (Scheme 1.15).¹¹⁴



Scheme 1.15

A literature search of synthetic stigmasterol derivatives shows that much of the work on this topic in the 1960's – 1990's is under patent, which adds further relevance to this area.

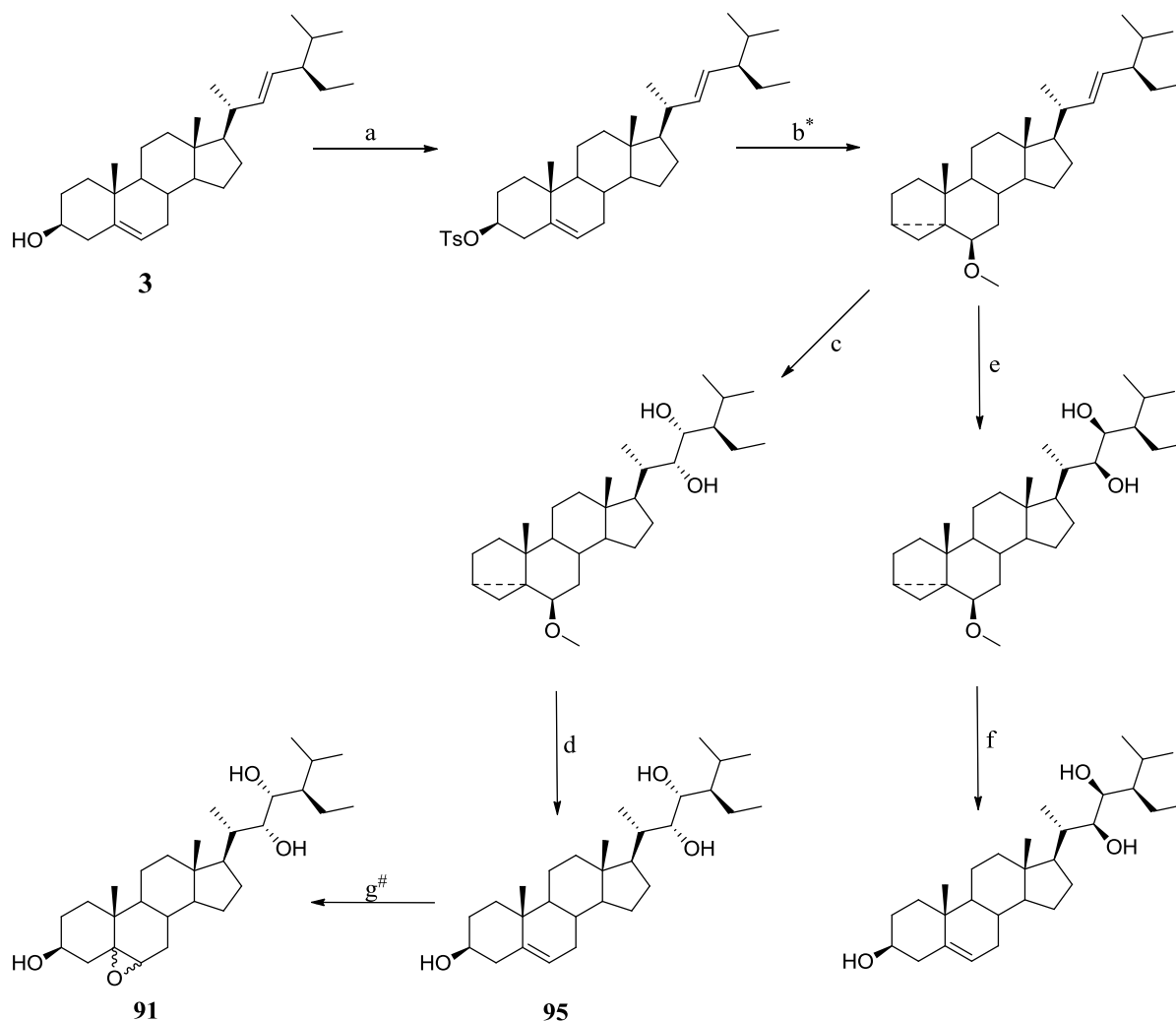
In 2010, the synthesis of eight oxides of stigmasterol (**88** – **95**) was described by Foley *et al.*¹¹⁵ Scheme 1.16 below describes the synthesis of six of these eight isolates, through a variety of protections, oxidations, epoxidations, ring openings and deprotections.



a = 0.95 eq *m*-CPBA, DCM (77%); b = Ac₂O, pyridine (89%); c = KMnO₄-CuSO₄·5H₂O, *t*-BuOH, H₂O (49%); d = Na₂CO₃, MeOH (92%); e = 3.7 eq *m*-CPBA, DCM (54%); f = H₂SO₄, THF-H₂O (32%) g = CrO₃, dimethylpyrazole, DCM -20 to 5 °C (66%); h = K₂CO₃, MeOH (59%); i = CeCl₃·7H₂O, NaBH₄, MeOH (54%).

Scheme 1.16

The final two of the eight stigmasterol oxides described by Foley *et al.*¹¹⁵ are synthesised according to Scheme 1.17, shown below. In this case, the protection of the C-5,6 alkene is required in order to facilitate chemistry of the side chain alkene at C-22,23. The protection reaction described by Foley will be utilised in this project and will be seen in Chapter 2.



a = *p*-TsCl, DMAP, pyridine (94%); b = MeOH (anhydrous), pyridine, (60%), * the side product of the solvolysis reaction is removed from the scheme for clarity; c = OsO₄, NMO, *t*-BuOH, THF, H₂O (47%); d = H₂SO₄, THF, H₂O (87%); e = OsO₄, NMO, DHQD PHN (dihydroquinidine 9'-phenanthryl ether), *t*-BuOH, THF, H₂O (30%); f = H₂SO₄, THF, H₂O (67%); g = *m*-CPBA, DCM (45%) [#] formed as a 5:1 mixture of the α and β epoxides. This scheme is adapted from Foley *et al.*,¹¹⁵ side products and unsuccessful reactions are removed for clarity.

Scheme 1.17

All eight compounds were strategically synthesised for comparison with the most toxic β -sitosterol oxidation products described by McCarthy *et al.*,¹¹⁶ which will be discussed in the following section. This study was moved forward by exploiting the additional sites for oxidation, due to the presence of the second alkene bond on the side chain of stigmasterol. Through a variety of selective epoxidations and oxidations, a number of epoxide, alcohol and ketone derivatives of stigmasterol were synthesised.

One of the challenges associated with the analysis of phytosterol oxidation products is that much of the studies available in the literature have been carried out on phytosterol blends,^{117,118} primarily because the phytosterols are readily available as mixtures with other plant sterols, namely β -sitosterol and campesterol. The advantage of this study is that the series of stigmasterol oxides were synthesised in a pure state, and that their biological characteristics can be evaluated accurately.

The percentage of viable cells following a 24 hour incubation period with each of the eight oxidised derivatives of stigmasterol was determined in the U937 cell line using the Fluorescein Diacetate/Ethidium Bromide (FDA/EtBr) staining assay. This assay will also be used to determine the biological activity of compounds synthesised in our research, as will be discussed in Chapter 2. The results following exposure to 30 μ M, 60 μ M or 120 μ M of each compound are presented below (Table 1.5).

Table 1.5 Percentage viable U937 cells following a 24 h exposure to stigmasterol oxides

Entry	Compound	30 μ M	60 μ M	120 μ M
1	88	96.8 \pm 1.7	96.0 \pm 1.5	94.0 \pm 0.6
2	89	98.0 \pm 1.5	96.3 \pm 1.4	96.3 \pm 0.9
3	90	51.4 \pm 8.3	39.6 \pm 4.6	7.6 \pm 2.2
4	91	95.4 \pm 1.5	23.3 \pm 3.4	3.6 \pm 0.7
5	92	95.7 \pm 2.0	96.7 \pm 0.7	93.7 \pm 1.8
6	93	85.8 \pm 4.0	56.3 \pm 6.5	22.8 \pm 0.6
7	94	97.7 \pm 0.6	95.0 \pm 1.5	92.3 \pm 3.5
8	95	75.0 \pm 2.8	63.3 \pm 13.0	52.3 \pm 4.1

Note: Cell viability in untreated control cells was 97.1 \pm 1.0%

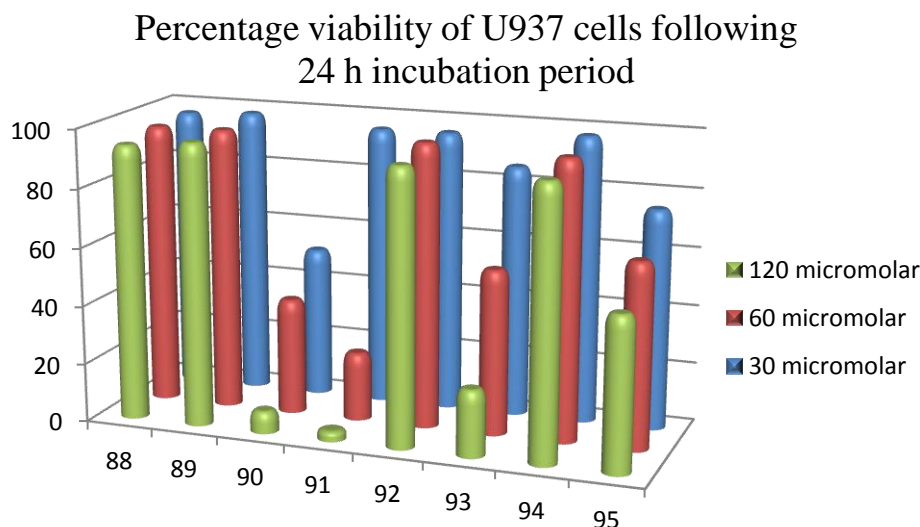


Figure 1.23

The diepoxide **90** (Table 1.5, Entry 3) showed the lowest percentage viability of the U937 cells across the 30 μM and 60 μM range of concentrations, and therefore is the most cytotoxic of the compounds screened. Interestingly, at a concentration of 120 μM , the epoxydiol **91** is slightly more active than the diepoxide **90**, having a percentage viability of 3.6% compared to 7.6%. Another interesting trend seen across the compounds screened is that both the α and β epoxides **88** and **89**, the 7-keto derivative **92** and the 3,5,6 triol **94** show very poor biological activity across all concentration values, each having percentage viabilities in the high 90's.

The cytotoxicity of each of these compounds was also examined in terms of apoptosis, using the MTT assay. Unlike the FDA/EtBr staining method, which quantifies the number of live cells in a population, this assay reflects cell proliferation and metabolic activity, which may be increased or decreased under certain conditions, such as the addition of chemical compounds. Each of the oxides of stigmaterol was tested for its ability to induce apoptosis by a morphological examination of the cell nuclei following staining with Hoechst 33342. In apoptotic nuclei, the DNA is cleaved into regular sized fragments that appear sufficiently distinctive to allow visual quantification in a sample.¹¹⁹

Table 1.6 Percentage apoptotic nuclei following 24 h incubation with stigmasterol oxides

Entry	Compound	30 μ M	60 μ M	120 μ M
1	88	2.6 ± 0.2	4.1 ± 1.4	4.4 ± 0.9
2	89	2.9 ± 1.1	3.6 ± 1.1	4.8 ± 0.1
3	90	43.9 ± 2.5	36.8 ± 4.3	21.6 ± 2.4
4	91	6.4 ± 0.9	29.3 ± 3.0	25.3 ± 5.0
5	92	5.1 ± 0.6	3.5 ± 0.2	3.9 ± 0.4
6	93	9.6 ± 1.1	20.3 ± 2.2	19.8 ± 3.8
7	94	2.6 ± 0.3	2.7 ± 1.2	3.0 ± 0.9
8	95	1.7 ± 0.9	2.7 ± 0.6	3.0 ± 0.5

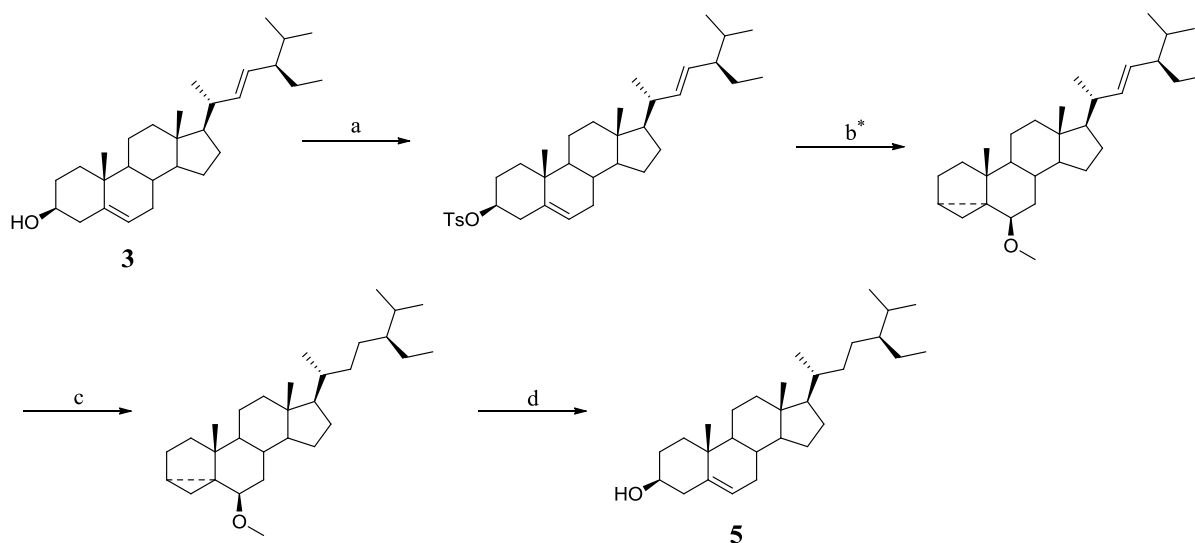
A comparable trend was observed for this assay, in that the relative toxicities of each of the compounds were similar. The diepoxide **90** shows a significant increase in the percentage of apoptotic cells at all concentrations tested, relative to the untreated control cells. It is interesting to note that increasing concentrations of stigmasterol oxide did not correlate with an increase in apoptosis in all samples. An example of this is in the case where cells were exposed to the diepoxide **90**. In this case the level of apoptosis was highest at the lowest concentration, indicating that at higher concentrations cells are more likely to die by necrosis.^{120,121}

In an interesting contrast to the findings by Ryan *et al.*, namely the apoptosis induced by both the 7-keto derivatives of cholesterol and sitosterol,¹²² there was no evidence of apoptosis for the 7-keto derivative of stigmasterol **92**.

1.12 Synthetic β -Sitosterol Derivatives and their Biological Activities

As mentioned in the previous section, the synthesis of stigmasterol oxides was preceded by a study on β -sitosterol **5** and its oxidation products, both their synthesis and biological activity.¹¹⁶ This study is closely comparable to that of the stigmasterol oxides, in its objectives, chemical transformations and biological evaluation. β -Sitosterol is the most prevalent phytosterol synthesised in plants, and given its structural similarity to cholesterol, can undergo oxidation by the same means as cholesterol. Therefore, for the reasons outlined at the beginning of the previous section, a study of the biological activity of β -sitosterol oxides is an important research concern.

McCarthy *et al.* present an attractive route for the synthesis of β -sitosterol from stigmasterol **3** (Scheme 1.18).¹¹⁶ The product is formed in 98.9% purity and with moderate to high yields across the four synthetic steps.

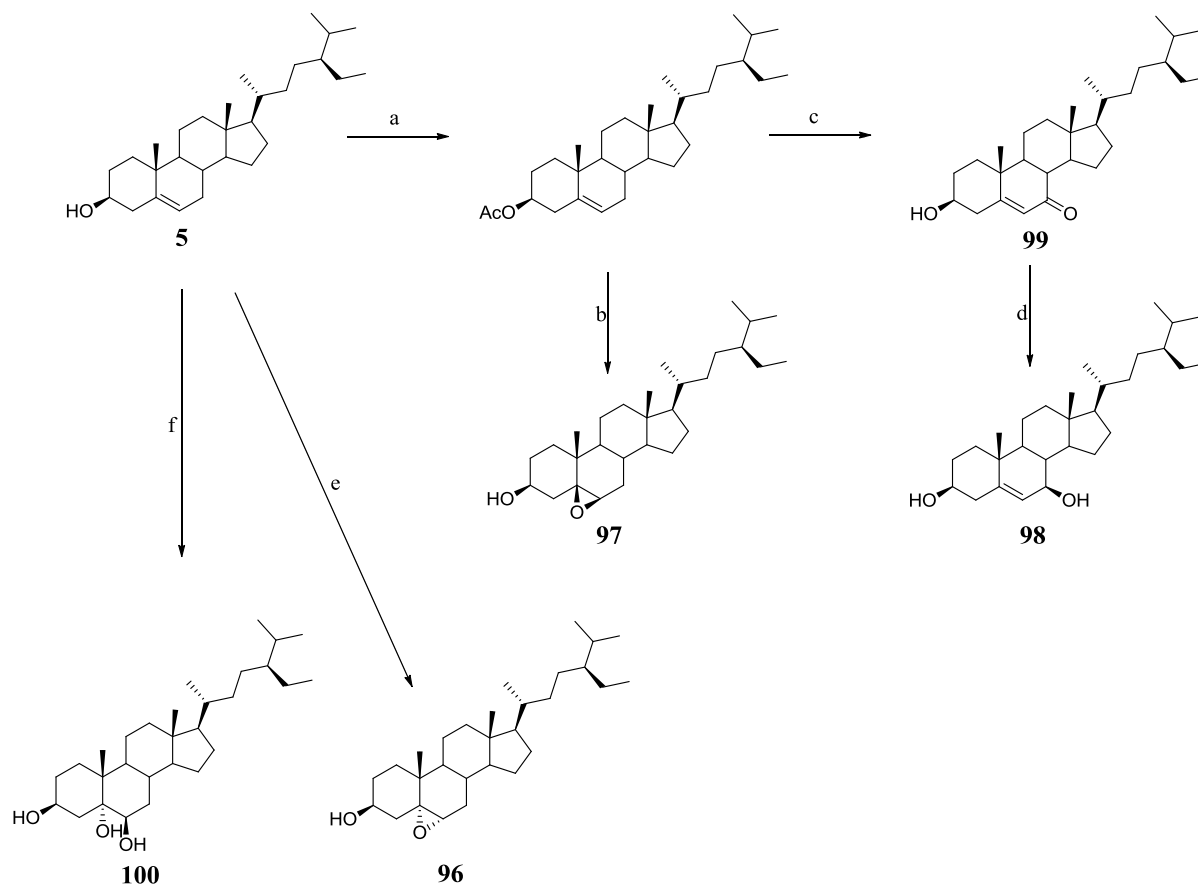


a = *p*-TsCl, DMAP, pyridine (90%); b = MeOH (anhydrous), pyridine (74%) * the side product of the solvolysis reaction is removed from the scheme for clarity; c = H₂, Pd/C 5%, EtOH (55%); d = *p*-TsOH, aq. Dioxane, 80 °C (55%).

Scheme 1.18

Upon synthesis of β -sitosterol in a pure state, several β -sitosterol oxidation products (**96** – **100**) were chosen as synthetic targets for biological evaluation. The synthesis of the oxides of β -sitosterol was carried out by manipulation of the C-5,6 alkene bond, incorporating allylic

oxidations and epoxidation chemistry, and using an acetate protecting group for the C-3 alcohol where necessary (Scheme 1.19).



a = Ac_2O , pyridine (88%); b = $\text{CuSO}_4/\text{KMnO}_4$, *t*-BuOH, H_2O (54%), followed by K_2CO_3 , MeOH (71%); c = CrO_3 , dimethylpyrazole, DCM, -20 to 5°C (79%), followed by K_2CO_3 , MeOH (91%); d = $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$, NaBH_4 (87%); e = *m*-CPBA, DCM, 0°C (70%); f = HCOOH , 80°C , 10 min, followed by H_2O_2 , followed by MeOH, NaOH (49% for three steps).

Scheme 1.19

These five compounds (**96** – **100**) were evaluated for their cytotoxicity in U937 and CaCo-2 cells. The percentage of viable cells following a 24 hour incubation period with the five oxidised derivatives of β -sitosterol was determined using the Fluorescein Diacetate/Ethidium Bromide (FDA/EtBr) staining assay. Although these compounds showed poor activity in the U937 cell line,¹²² an improvement in cytotoxicity was seen in the CaCo-2 cell line (Table 1.7 and Figure 1.24).

Table 1.7 Percentage viable CaCo-2 cells following a 24 h exposure to β -sitosterol oxides

Entry	Compound	30 μ M	60 μ M	120 μ M
1	96	102.6 \pm 1.2	101.1 \pm 2.2	101.1 \pm 4.4
2	97	111.7 \pm 3.5	110.2 \pm 4.7	109.0 \pm 0.6
3	98	91.6 \pm 11.7	61.9 \pm 9.1	40.8 \pm 4.0
4	99	93.0 \pm 4.7	64.4 \pm 7.0	23.3 \pm 4.2
5	100	97.1 \pm 0.9	78.6 \pm 9.2	54.8 \pm 13.6

Percentage viability of CaCo-2 cells following 24 h incubation period

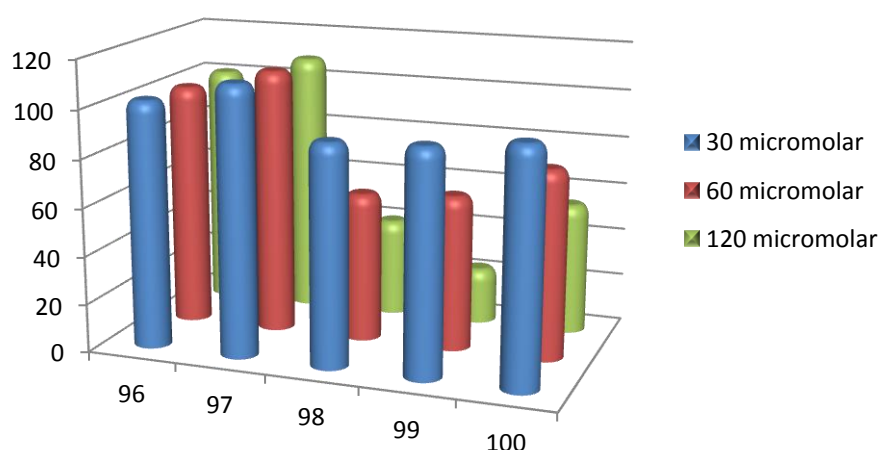


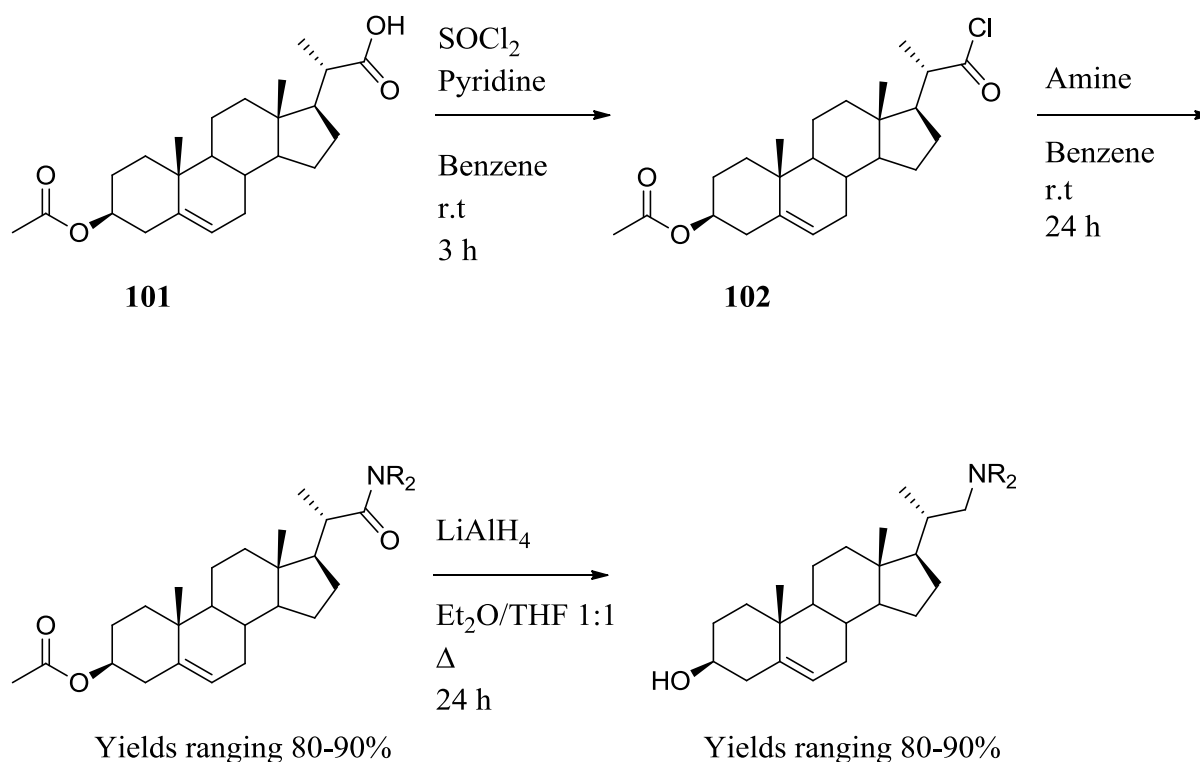
Figure 1.24

While both α and β epoxides, **96** and **97**, were found to be inactive in the CaCo-2 cell line as well as the U937 cell line, there was an improvement in cytotoxicity of the other three compounds (**98** – **100**) at a concentration of 120 μ M. The standout result from this biological screen was the cytotoxicity of the 7-keto derivative of β -sitosterol **99**, giving a percentage viability of CaCo-2 cells of 23.3% at 120 μ M. By analysis of the results from the stigmaterol and β -sitosterol studies, a conclusion can be drawn that there is greater potential for cytotoxic activity in the stigmaterol oxides. This result is appreciable given the possibility of further functionalisation of the stigmaterol moiety by exploiting the additional alkene present in the side chain of the structure. Therefore, novel derivatives of stigmaterol would warrant attention in further studies, in the expectation that these compounds would prove significantly cytotoxic against the U937 cell line.

1.13 Aminosteroids and Amidosteroids

Steroidal compounds containing various nitrogen functionalities, including primary, secondary and tertiary amines and amides, are reported in the literature, mainly as antifungal agents.¹²³⁻¹²⁶

Preliminary work in this field was carried out by James *et al.*¹²⁷ and Dodgson *et al.*¹²⁸ who prepared amino derivatives of cholesterol. These derivatives were examined as antibacterial agents or amoebicides and found to be not particularly effective. Herzog *et al.* developed this area by synthesising a range of amides and amines of a cholestane nature.¹²³ The methodology employed to synthesise the derivatives, shown in Scheme 1.20, involved conversion of Δ^5 -3 β -acetoxybisanorcholeonic acid **101** to the corresponding acid chloride **102**, followed by subsequent amidation and reduction to yield the amides and amines respectively.



Scheme 1.20

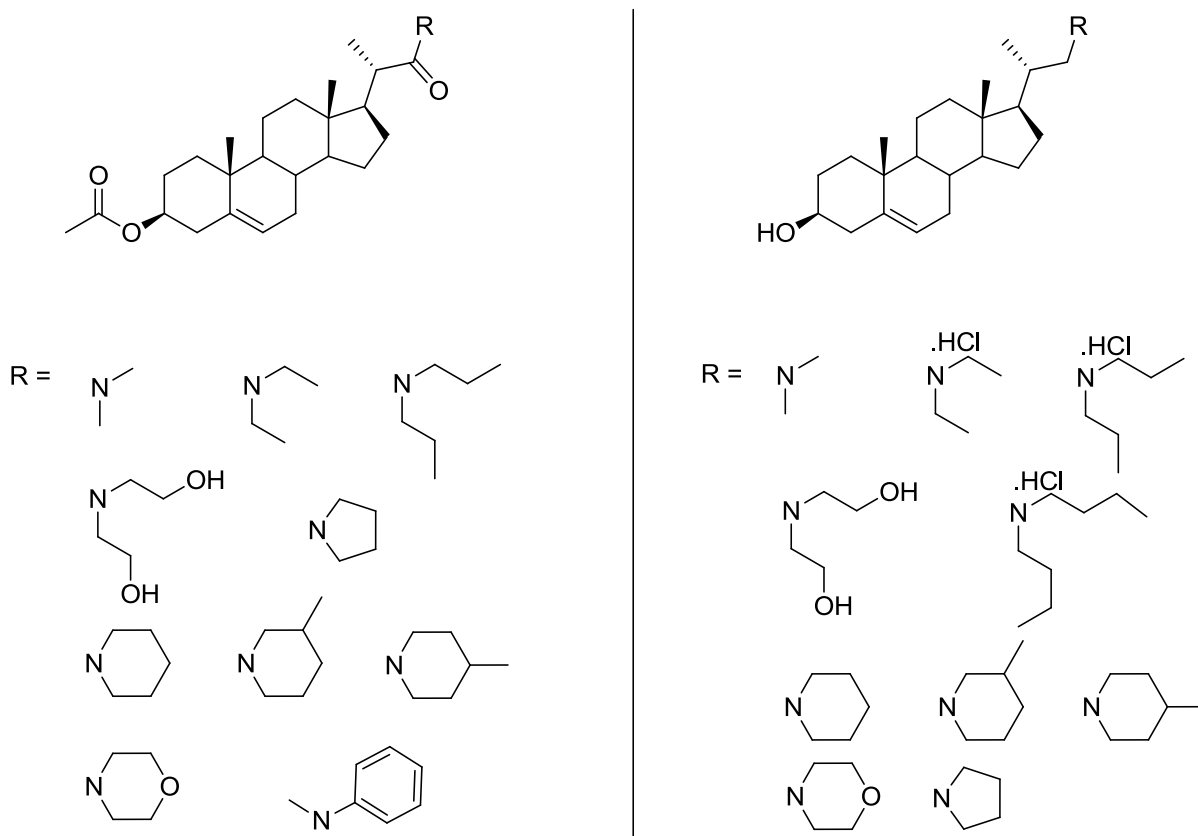
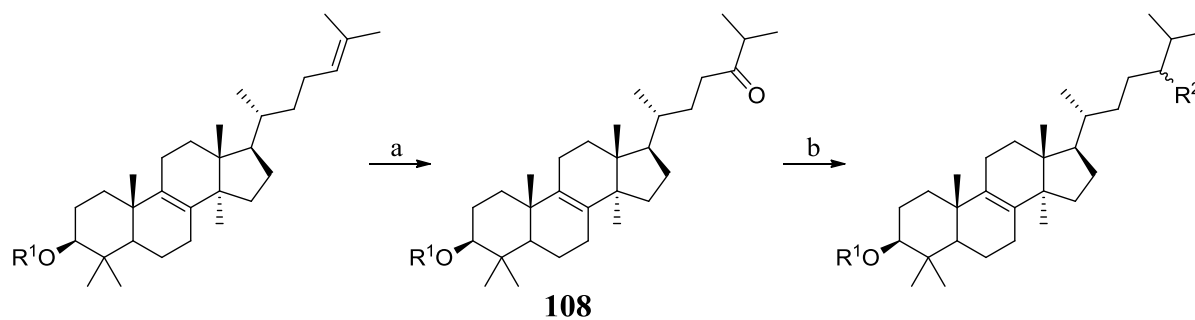


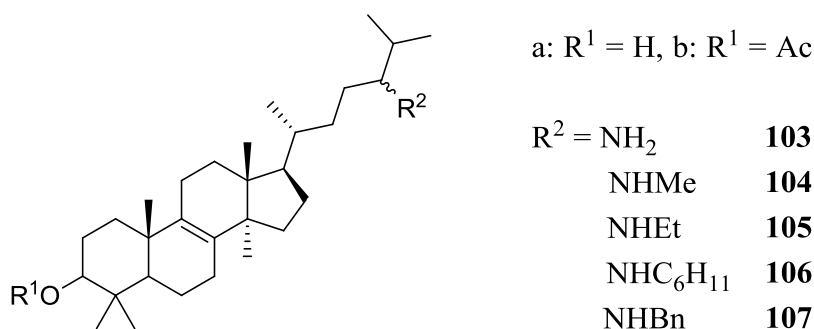
Figure 1.25. R groups for amide and amine derivatives shown in Scheme 1.20

None of the amide derivatives shown in Figure 1.25 possessed significant activity, however the amines were effective in inhibiting the fungus *Candida albicans*. The piperidino and pyrrolidino derivatives were the most effective of these amines, exhibiting activity at concentrations as low as 0.1 – 1.0 mg/mL.

Antifungal activity is also displayed in the amine derivatives of the lanostane nucleus (**103** – **107**), as reported by Chung *et al.*¹²⁴ The derivatives are characterised by various amine moieties as substituents at C-24, and were synthesised by initially treating lanosterol (or lanosterol acetate) with *m*-CPBA followed by BF₃·OEt₂ affording 24-keto lanosterol **108** in 43% overall yield (Scheme 1.24).¹²⁹ This was followed by subsequent reductive amination with the appropriate amine, zinc chloride and sodium cyanoborohydride to synthesise **104** – **107** in moderate yields.¹³⁰



a = *m*-CPBA, DCM, r.t, 3 h, then BF₃·OEt₂, DCM, r.t, 10 min (43%); b = amine, NaBH₃CN, ZnCl₂, MeOH, r.t, 16 h, (42% yield for methylamine derivative {representative procedure reported by Chung} other yields unreported)



Scheme 1.24

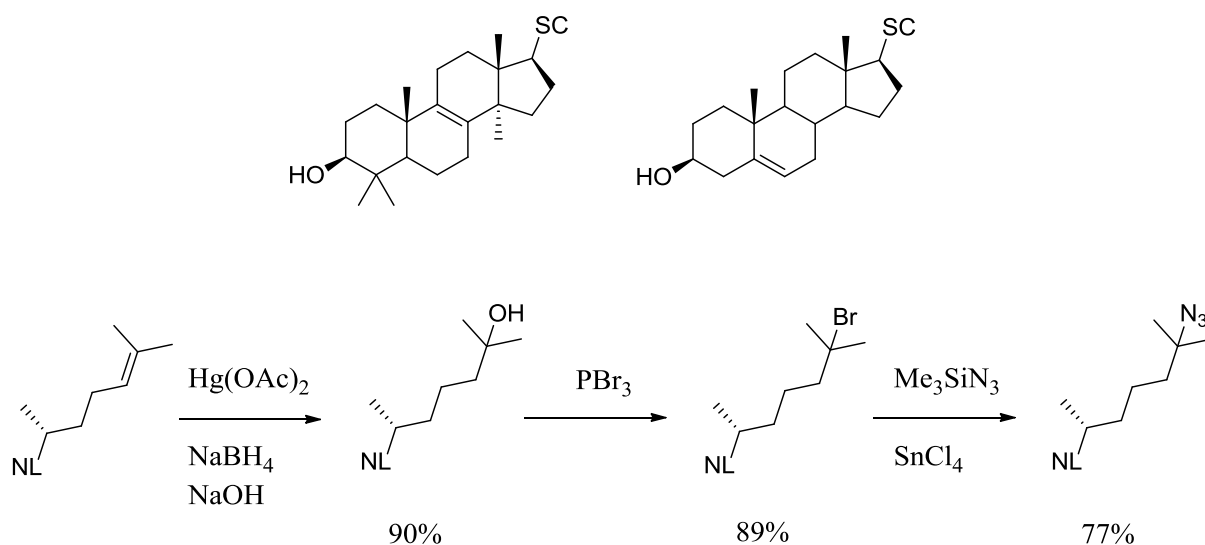
The antifungal properties of these amines were evaluated against several fungal strains as shown below in Table 1.8.

Table 1.8 *in vitro* Antifungal Activities (μg/mL)

Entry	Fungal Strain	103a	104a	104b	105a	105b	106a	107a
1	<i>C. albicans</i>	100	100	100	25	100	50	100
2	<i>C. krusei</i>	100	100	100	50	100	100	100
3	<i>C. parapsilosis</i>	100	100	100	100	100	100	100
4	<i>C. pseudotropicalis</i>	100	100	100	100	100	100	100
5	<i>Cr. neoformans</i>	0.10	0.78	100	25	50	12.5	3.13
6	<i>S. carlsbergensis</i>	25	6.25	100	25	50	50	50
7	<i>S. cerevisiae</i>	25	6.25	100	12.5	100	50	50
8	<i>T. candida</i>	100	100	100	100	100	100	100
9	<i>C. glabrata</i>	50	25	100	25	100	100	100

Clear trends in this series of results are noteworthy. Firstly, the C-3 alcohol derivatives show higher antifungal activities than their corresponding C-3 acetate analogues. Secondly, the methyl amino derivative (**104a**) generally displayed improved activity against several fungal strains than its other amine counterparts (Table 1.8, Entries 5, 6, 7, 9).

Beuchet *et al.* progressed the work of Chung by investigating the impact of a C-25 aminosterol on antifungal activity.¹²⁵ Synthesis of C-25 amino lanostane and cholestane compounds (**108** and **109**) proceeded *via* the hydroxylation of the C-25 position, followed by bromination, azidotisation and reduction to yield the desired aminosterols in good yield.¹³¹



Notes: NL = Nucleus, SC = Side chain, reduction of the azide species yielded compounds shown below (Figure 1.26) in “good” yields.

Scheme 1.22

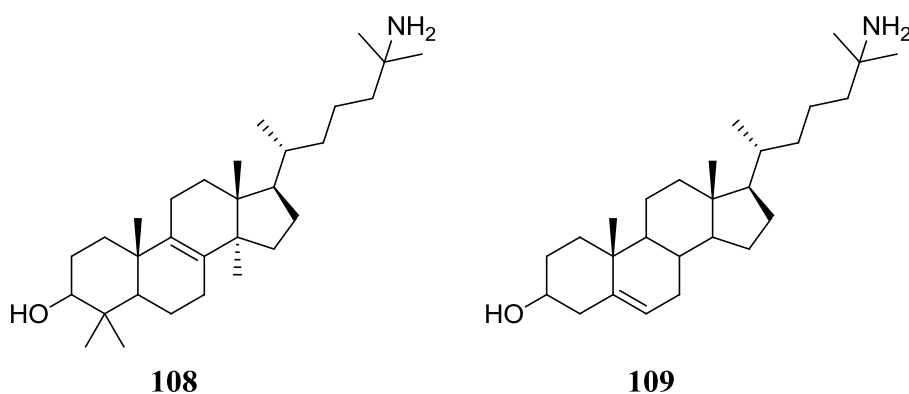


Figure 1.26

Both of these amines were found to inhibit *Candida albicans*, with the cholestane derivative showing excellent growth inhibition at 4 μ M. These results are in perfect concordance with the reported fungicidal activity of 24-aminocholesterol and 24-aminolanosterol reported by Chung *et al.*¹²⁴

1.14 Amines in Pharmaceutical Compounds

The role of amines in pharmaceuticals is immense, and typical functions of amine based drugs are stimulants, vasoconstrictors, decongestants, analgesics, antihistamines and antidepressants.

Perhaps the most recognized pharmaceutical amine is morphine **110**, a potent alkaloid analgesic drug used to relieve severe pain. In clinical medicine, morphine is regarded as the benchmark of opioid analgesics for the relief of chronic pain.¹³² As with other opioids, morphine acts directly on the central nervous system to reduce pain. Its duration of analgesia is approximately four hours when administered *via* the intravenous or intramuscular route and approximately six hours when administered orally. Although morphine is a natural substance, isolable from the poppy plant, the synthesis of morphine has been reported in the literature dating back to 1952.¹³³⁻¹⁴² While morphine exhibits potent analgesic properties, excess intake of the drug can lead to asphyxiation and death. Therefore, milder drugs or “morphine substitutes” are often employed for pain relief.

Codeine **111**, or 3-methyl morphine, is also used as an analgesic to treat mild to moderate pain. It is marketed either as a single ingredient drug, or as a combination preparation with paracetamol, ibuprofen or aspirin. By comparison with morphine, the adverse effects of codeine are far less serious, with the most common side effect being drowsiness. However, tolerance to the side effects of codeine develops with prolonged use, and addiction to the substance becomes an issue.¹⁴³ In the Republic of Ireland, codeine remains semi non-prescriptive up to a limit of 12.8 milligrams per tablet. However, regulations introduced in August 2010 requires that these products are “not accessible to the public for self selection”, meaning the patient must discuss their pain relief requirements with a pharmacist prior to obtaining products containing codeine. [Source: “Codeine regulations cause a few Headaches” *The Irish Times* 24/08/2010]

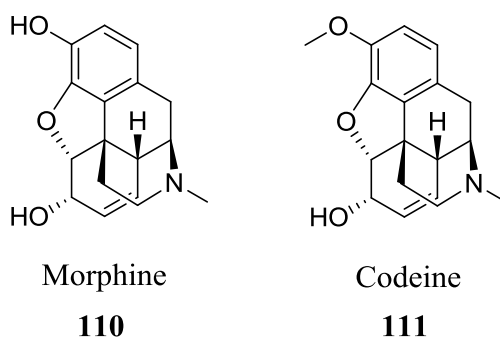
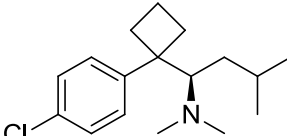
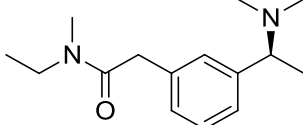
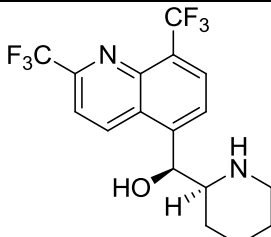
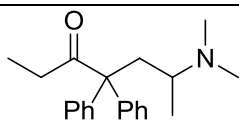
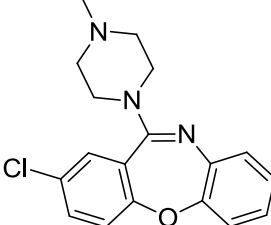
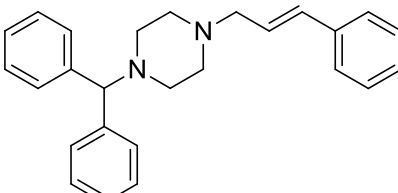


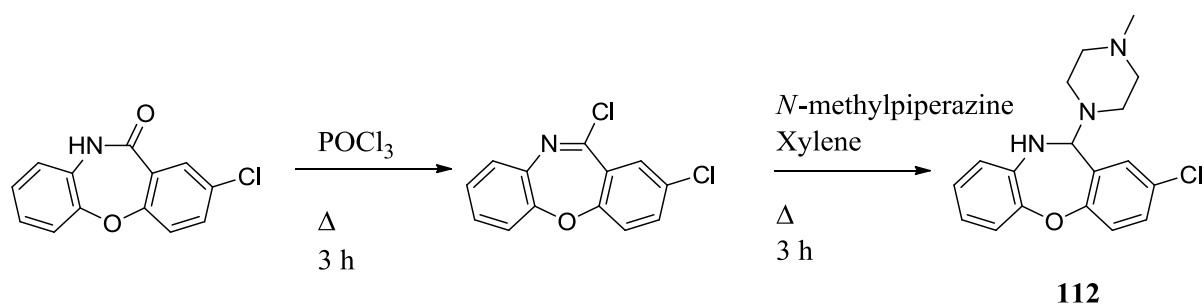
Figure 1.27

A selection of other pharmaceuticals containing amine functionality is detailed in Table 1.9. Possibly the most well known of these drugs is the antihistamine cinnarizine, marketed as Stugeron[®], and used to combat nausea due to motion sickness or vertigo.¹⁴⁴

Table 1.9

Entry	Name	Structure	Purpose	Manufacturer
1	Sibutramine		Obesity	Abbott
2	Rivastigmine		Alzheimers	Novartis
3	Lariam		Malaria	Roche
4	Methadone		Analgesic	Generic
5	Loxapine		Schizophrenia	Alexza
6	Cinnarizine		Antihistamine	Janssen

Loxapine **112** is a first-generation antipsychotic that has been reformulated into an inhaled powder, which allows for direct administration to the lungs. Inhaled Loxapine at a 10 mg dose was approved by the US Food and Drug Administration in December 2012 for the treatment of agitation associated with schizophrenia and bipolar disorder in adults.¹⁴⁵ The synthesis of Loxapine is described in US patent 3,546,226 (Scheme 1.23 below).¹⁴⁶



Scheme 1.23

1.15 Conclusion

In summary, steroidal compounds are known to be hugely important, both in terms of regulating biological events in the human body, and as pharmaceuticals. Their multiple alkene functionalities make them suitable for modification by conventional means, and allow a range of functional groups to be synthesised at strategic positions.

Lanosterol and stigmasterol were the precursors chosen for this work due to their structural features and the fact that they are readily available. Literature precedent for the synthesis of lanosterol oxidation products is widely available, as seen in the anti-tumour properties of *Antrodia camphorata* isolates.⁴⁹ Functional group transformations which are similar to those described in Figure 1.22 will be carried out in this research. Namely the formation of acetate and ketone groups at C-3 by manipulation of the alcohol moiety at this position, allylic oxidations and epoxidations at the C-8,9 alkene to synthesise epoxides and ketones, and side chain derivitisation by means of epoxidations, oxidative cleavage and reductive amination. The antifungal activity of many amine derivatives of the cholestane and lanostane nucleus provides incentive for the elaboration of this work with the view to evaluating these types of compounds for anti-cancer activity.¹²³⁻¹²⁵ Compounds synthesised will be biologically evaluated for cytotoxicity against CaCo-2 and U937 cell lines using the MTT assay, and the results will be presented in terms of percentage viability of cells and IC₅₀ results. In addition, the apoptosis potential of the compounds is presented in terms of a fold increase relative to the ethanol carrier control.

The research described herein undertakes the synthesis of novel lanosterol oxidation products as well as side chain amine derivatives of lanosterol and stigmasterol. Literature precedent for manipulation at key sites in the steroids has been described in Chapter 1, and the results of the research carried out in this project will be discussed in Chapter 2. The biological evaluation of these compounds forms part of the process of the strategic design of successful derivatives, and these evaluations will be performed at intermediate stages throughout the research in order to provide an insight into the most cytotoxic functional groups.

References

1. Shoppee, C. W. *Chemistry of the Steroids*; Butterworths, London: **1964**.
2. Hill, R. A. *Dictionary of Steroids*; Chapman and Hall London: **1991**.
3. Hill, R. A.; Connolly, J. D. *Nat. Prod. Rep.* **2013**, *30*, 1028-1065.
4. Petronelli, A.; Pannitteri, G.; Testa, U. *Anti-Cancer Drugs* **2009**, *20*, 880-892.
5. Huang, M.; Lu, J. J.; Huang, M. Q.; Bao, J. L.; Chen, X. P.; Wang, Y. T. *Expert Opinion on Investigational Drugs* **2012**, *21*, 1801-1818.
6. Connolly, J. D.; Hill, R. A. *Nat. Prod. Rep.* **2010**, *27*, 79-132.
7. Bishayee, A.; Ahmed, S.; Brankov, N.; Perloff, M. *Frontiers in Bioscience-Landmark* **2011**, *16*, 980-996.
8. Connolly, J. D.; Hill, R. A. *Nat. Prod. Rep.* **2008**, *25*, 794-830.
9. Johns, W. F. *Steroids*; Butterworths, London: **1973**.
10. Risley, J. M. *J. Chem. Educ.* **2002**, *79*, 377-384.
11. Buyuktuncer, Z.; Fisunoglu, M.; Guven, G. S.; Unal, S.; Besler, H. T. *Lipids Health Dis.* **2013**, *12*.
12. Frye, L. L.; Leonard, D. A. *Crit. Rev. Biochem. Mol.* **1999**, *34*, 123-140.
13. Pereira, M.; Song, Z. H.; Santos-Silva, L. K.; Richards, M. H.; Nguyen, T. T. M.; Liu, J. L.; Soares, C. M. D.; Cruz, A. H. D.; Ganapathy, K.; Nes, W. D. *Bba-mol. Cell. Biol. L.* **2010**, *1801*, 1163-1174.
14. Brunt, S. A.; Riehl, R.; Silver, J. C. *Mol. Cell. Biol.* **1990**, *10*, 273-281.
15. Jin, H.; McCaffery, J. M.; Grote, E. *J. Cell Biol.* **2008**, *180*, 813-826.
16. Ryan, E.; McCarthy, F. O.; Maguire, A. R.; O'Brien, N. M. *Food. Rev. Int.* **2009**, *25*, 157-174.
17. Sanclemente, T.; Marques-Lopes, I.; Puzo, J.; Garcia-Otin, A. L. *J. Physiol. Biochem.* **2009**, *65*, 87-98.
18. Woyengo, T. A.; Ramprasath, V. R.; Jones, P. J. H. *Eur. J. Clin. Nutr.* **2009**, *63*, 813-820.
19. Tilvis, R. S.; Miettinen, T. A. *Am. J. Clin. Nutr.* **1986**, *43*, 92-97.
20. Weingartner, O.; Bohm, M.; Laufs, U. *Deut. Med. Wochenschr.* **2008**, *133*, 1201-1204.
21. Weingartner, O.; Luetjohann, D.; Ji, S. B.; Weisschoff, N.; List, F.; Sudhop, T.; von Bergmann, K.; Gertz, K.; Konig, J.; Schafers, H. J.; Endres, M.; Bohm, M.; Laufs, U. *J. Am. Coll. Cardiol.* **2008**, *51*, 1553-1561.
22. Cenedella, R. J. *Lipids* **1982**, *17*, 443-447.
23. Dias, J. R.; Gao, H. W. *Spectrochim. Acta A.* **2009**, *74*, 1064-1071.
24. Ryan, K. J. *Cancer Res.* **1982**, *42*, 3342-3344.
25. Mooradian, A. D.; Morley, J. E.; Korenman, S. G. *Endocr. Rev.* **1987**, *8*, 1-28.
26. Reiterreimers, M.; Baltes, W. Z. *Lebensm. Unters. For.* **1986**, *183*, 186-190.
27. Reiterreimers, M.; Baltes, W. Z. *Lebensm. Unters. For.* **1986**, *183*, 191-192.
28. Baltes, W.; Hirsemann, T. Z. *Lebensm. Unters. For.* **1986**, *183*, 193-198.
29. Guardiola, F. *Cholesterol and Phytosterol Oxidation Products: Analysis, Occurrence and Biological Effects*; AOCS Publishing, Illinois: **2002**.
30. Bittman, R. *Cholesterol: Its Functions and Metabolism in Biology and Medicine*; Springer, New York: **1997**.
31. Pirillo, A.; Norata, G. D.; Catapano, A. L. *Curr. Pharm. Des.* **2013**, *19*, 3841-3857.
32. Grattan, B. J. *Nutrients* **2013**, *5*, 359-387.
33. Cliff, W. J. *Coronaries and Cholesterol*; Chapman and Hall: 1989.
34. Vanmierlo, T.; Husche, C.; Schott, H. F.; Pettersson, H.; Lutjohann, D. *Biochimie* **2013**, *95*, 464-472.
35. Nes, W. R. *Lipids* **1974**, *9*, 596-612.
36. Piironen, V.; Lindsay, D. G.; Miettinen, T. A.; Toivo, J.; Lampi, A. M. *J. Sci. Food Agr.* **2000**, *80*, 939-966.
37. Sundararaman, P.; Djerassi, C. *J. Org. Chem.* **1977**, *42*, 3633-3634.
38. Weihrach, J. L.; Gardner, J. M. *J. Am. Diet. Assoc.* **1978**, *73*, 39-47.
39. Brown, A. W.; Hang, J. L.; Dussault, P. H.; Carr, T. P. *Lipids* **2010**, *45*, 855-862.
40. Wetherbee, B. M.; Nichols, P. D. *Comp. Biochem. Phys. B.* **2000**, *125*, 511-521.

41. Hernandez-Perez, M.; Gallego, R. M. R.; Alayon, P. J. P.; Hernandez, A. B. *Mar. Freshwater Res.* **1997**, *48*, 573-576.
42. Vantamel, EE; Willett, J. D.; Clayton, R. B. *J. Am. Chem. Soc.* **1967**, *89*, 3371.
43. Kongduang, D.; Wungsintaweekul, J.; De Eknankul, W. *Tetrahedron Lett.* **2008**, *49*, 4067-4072.
44. Dinda, B.; Debnath, S.; Mohanta, B. C.; Harigaya, Y. *Chemistry & Biodiversity* **2010**, *7*, 2327-2580.
45. Kuo, R. Y.; Qian, K. D.; Morris-Natschke, S. L.; Lee, K. H. *Nat. Prod. Rep.* **2009**, *26*, 1321-1344.
46. Rios, J. L.; Andujar, I.; Recio, M. C.; Giner, R. M. *J. Nat. Prod.* **2012**, *75*, 2016-2044.
47. Li, Y. Y.; Mi, Z. Y.; Tang, Y.; Wang, G.; Li, D. S.; Tang, Y. J. *Helv. Chim. Acta* **2009**, *92*, 1586-1593.
48. Sun, I. C.; Kashiwada, Y.; Morris-Natschke, S. L.; Lee, K. H. *Current Topics in Medicinal Chemistry* **2003**, *3*, 155-169.
49. Male, K. B.; Rao, Y. K.; Tzeng, Y. M.; Montes, J.; Kamen, A.; Luong, J. H. T. *Chem. Res. Toxicol.* **2008**, *21*, 2127-2133.
50. Hsu, Y. L.; Kuo, Y. C.; Kuo, P. L.; Ng, L. T.; Kuo, Y. H.; Lin, C. C. *Cancer Lett.* **2005**, *221*, 77-89.
51. Liu, D. Z.; Liang, Y. C.; Lin, S. Y.; Lin, Y. S.; Wu, W. C.; Hou, W. C.; Su, C. H. *Biosci. Biotech. Bioch.* **2007**, *71*, 23-30.
52. Shen, Y. C.; Wang, Y. H.; Chou, Y. C.; Chen, C. F.; Lin, L. C.; Chang, T. T.; Tien, J. H.; Chou, C. J. *Planta Med.* **2004**, *70*, 310-314.
53. Song, T. Y.; Yen, G. C. *J. Agr. Food. Chem.* **2002**, *50*, 3322-3327.
54. Deng, J. Y.; Chen, S. J.; Jow, G. M.; Hsueh, C. W.; Jeng, C. J. *Chem. Res. Toxicol.* **2009**, *22*, 1817-1826.
55. Hseu, Y. C.; Yang, H. L.; Lai, Y. C.; Lin, J. G.; Chen, G. W.; Chang, Y. H. *Nutr. Cancer* **2004**, *48*, 189-197.
56. Male, K. B.; Crowley, S. M.; Collins, S. G.; Tzeng, Y. M.; Luong, J. H. T. *Anal. Methods* **2010**, *2*, 870-877.
57. Yeh, C. T.; Rao, Y. K.; Yao, C. J.; Yeh, C. F.; Li, C. H.; Chuang, S. E.; Luong, J. H. T.; Lai, G. M.; Tzeng, Y. M. *Cancer Lett.* **2009**, *285*, 73-79.
58. Giner-Larza, E. M.; Manez, S.; Giner-Pons, R. M.; Recio, M. C.; Rios, J. L. *J. Ethnopharmacol.* **2000**, *73*, 61-69.
59. Ukiya, M.; Akihisa, T.; Tokuda, H.; Hirano, M.; Oshikubo, M.; Nobukuni, Y.; Kimura, Y.; Tai, T.; Kondo, S.; Nishino, H. *J. Nat. Prod.* **2002**, *65*, 462-465.
60. Akihisa, T.; Nakamura, Y.; Tokuda, H.; Uchiyama, E.; Suzuki, T.; Kimura, Y.; Uchikura, K.; Nishino, H. *J. Nat. Prod.* **2007**, *70*, 948-953.
61. Akihisa, T.; Uchiyama, E.; Kikuchi, T.; Tokuda, H.; Suzuki, T.; Kimura, Y. *J. Nat. Prod.* **2009**, *72*, 1786-1792.
62. Nukaya, H.; Yamashiro, H.; Fukazawa, H.; Ishida, H.; Tsuji, K. *Chem. Pharm. Bull.* **1996**, *44*, 847-849.
63. Yasukawa, K.; Kaminaga, T.; Kitanaka, S.; Tai, T.; Nunoura, Y.; Natori, S.; Takido, M. *Phytochemistry* **1998**, *48*, 1357-1360.
64. Kamo, T.; Asanoma, M.; Shibata, H.; Hirota, M. *J. Nat. Prod.* **2003**, *66*, 1104-1106.
65. Zhou, L.; Zhang, Y. C.; Gapter, L. A.; Ling, H.; Agarwal, R.; Ng, K. Y. *Chem. Pharm. Bull.* **2008**, *56*, 1459-1462.
66. She, G. M.; Zhu, N. L.; Wang, S.; Liu, Y.; Ba, Y. Y.; Sun, C. Q.; Shi, R. B. *Chem. Cent. J.* **2012**, *6*.
67. Arpha, K.; Phosri, C.; Suwannasai, N.; Mongkolthanaruk, W.; Sodngam, S. *J. Agr. Food. Chem.* **2012**, *60*, 9834-9841.
68. Phosri, C.; Martin, M. P.; Sihanonth, P.; Whalley, A. S.; Watling, R. *Mycol. Res.* **2007**, *111*, 275-286.
69. Kim, K. H.; Moon, E.; Choi, S. U.; Kim, S. Y.; Lee, K. R. *J. Nat. Prod.* **2013**, *76*, 845-851.

70. Shiono, Y.; Matsuzaka, R.; Wakamatsu, H.; Muneta, K.; Murayama, T.; Ikeda, M. *Phytochemistry* **2004**, *65*, 491-496.
71. Kim, D. S.; Baek, N. I.; Oh, S. R.; Jung, K. Y.; Lee, I. S.; Kim, J. H.; Lee, H. K. *Arch. Pharm. Res.* **1997**, *20*, 201-205.
72. Ikeda, M.; Sato, Y.; Izawa, M.; Sassa, T.; Miura, Y. *Agr. Biol. Chem.* **1977**, *41*, 1539-1541.
73. Ikeda, M.; Niwa, G. I.; Tohyama, K.; Sassa, T.; Miura, Y. *Agr. Biol. Chem.* **1977**, *41*, 1803-1805.
74. Kubo, I.; Matsumoto, A.; Kozuka, M.; Wood, W. F. *Chem. Pharm. Bull.* **1985**, *33*, 3821-3825.
75. Suzuki, K.; Fujimoto, H.; Yamazaki, M. *Chem. Pharm. Bull.* **1983**, *31*, 2176-2178.
76. Takahashi, A.; Kusano, G.; Ohta, T.; Ohizumi, Y.; Nozoe, S. *Chem. Pharm. Bull.* **1989**, *37*, 3247-3250.
77. Doi, K.; Shibata, T.; Yokoyama, N.; Terasawa, H.; Matsuda, O.; Kashino, S. *J. Chem. Soc. Chem. Comm.* **1990**, 725-726.
78. Lu, Z. Q.; Chen, G. T.; Zhang, J. Q.; Huang, H. L.; Guan, S. H.; Guo, D. A. *Helv. Chim. Acta* **2007**, *90*, 2245-2250.
79. Pei, Y. G.; Wu, Q. X.; Shi, Y. P. *J. Chin. Chem. Soc.* **2007**, *54*, 1565-1572.
80. Nakamura, S.; Iwami, J.; Matsuda, H.; Mizuno, S.; Yoshikawa, M. *Tetrahedron* **2009**, *65*, 2443-2450.
81. Kim, Y. O.; Park, H. W.; Kim, J. H.; Lee, J. Y.; Moon, S. H.; Shin, C. S. *Life Sciences* **2006**, *79*, 72- 80.
82. Song, Y.; Hui, J.; Kou, W.; Xin, R.; Jia, F.; Wang, N.; Hu, F. Q.; Zhang, H. L.; Liu, H. S. *Current Microbiology* **2008**, *57*, 454-462.
83. Nakata, T.; Yamada, T.; Taji, S.; Ohishi, H.; Wada, S.; Tokuda, H.; Sakuma, K.; Tanaka, R. *Bioorgan. Med. Chem.* **2007**, *15*, 257-264.
84. Nakajima, Y.; Sato, Y.; Konishi, T. *Chem. Pharm. Bull.* **2007**, *55*, 1222-1226.
85. Lee, I. K.; Kim, Y. S.; Jang, Y. W.; Jung, J. Y.; Yun, B. S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6678-6681.
86. Taji, S.; Yamada, T.; In, Y.; Wada, S. I.; Usami, Y.; Sakuma, K.; Tanakaa, R. *Helv. Chim. Acta* **2007**, *90*, 2047-2057.
87. Taji, S.; Yamada, T.; Tanaka, R. *Helv. Chim. Acta* **2008**, *91*, 1513-1524.
88. Barton, D. H. R.; Lewis, D. A.; Mcghie, J. F. *J. Chem. Soc.* **1957**, 2907-2915.
89. Mcghie, J. F.; Palmer, P. J.; Rosenberger, M. *Chemistry & Industry* **1959**, 1221-1223.
90. Barton, D. H. R.; Mcghie, J. F.; Daniels, P. J. L.; Palmer, P. J. *J. Chem. Soc.* **1963**, 3675-3691.
91. Barton, D. H. R.; Hameed, A.; Mcghie, J. F. *Tetrahedron Lett.* **1965**, 4343-4346.
92. Bentley, T. J.; Mcghie, J. F.; Barton, D. H. R. *Tetrahedron Lett.* **1965**, 2497-2498.
93. Parvez, M.; Edwards, O. E.; Paryzek, Z. *Acta. Crystallogr. C.* **2007**, *63*, O249-O251.
94. Paryzek, Z. *Tetrahedron Lett.* **1976**, 4761-4762.
95. Paryzek, Z. *J. Chem. Soc. Perk. T 1* **1978**, 329-336.
96. Briggs, L. H.; Bartley, J. P.; Rutledge, P. S. *J. Chem. Soc. Perk. T 1* **1972**, 581-583.
97. Wada, S.; Tanaka, R. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2966-2969.
98. Honda, Y.; Honda, T.; Roy, S.; Gribble, G. W. *J. Org. Chem.* **2003**, *68*, 4991-4993.
99. Sato, Y.; Sonoda, Y. *Chem. Pharm. Bull.* **1981**, *29*, 356-365.
100. Davis, C. E.; Bailey, J. L.; Lockner, J. W.; Coates, R. M. *J. Org. Chem.* **2003**, *68*, 75-82.
101. Corey, E. J.; Kim, C. U. *Tetrahedron Lett.* **1974**, 287-290.
102. Plietker, B. *Tetrahedron: Asymmetry* **2005**, *16*, 3453-3459.
103. Moreau, R. A.; Whitaker, B. D.; Hicks, K. B. *Prog. Lipid Res.* **2002**, *41*, 457-500.
104. Zhang, X.; Julien-David, D.; Miesch, M.; Geoffroy, P.; Raul, F.; Roussi, S.; Aoude-Werner, D.; Marchioni, E. *Steroids* **2005**, *70*, 896-906.
105. Johnsson, L.; Dutta, P. C. *J. Chromatogr. A* **2005**, *1064*, 213-217.
106. Conchillo, A.; Cercaci, L.; Ansorena, D.; Rodriguez-Estrada, M. T.; Lercker, G.; Astiasaran, I. *J. Agr. Food. Chem.* **2005**, *53*, 7844-7850.
107. Lampi, A. M.; Juntunen, L.; Toivo, J.; Piironen, V. *J. Chromatogr. B.* **2002**, *777*, 83-92.
108. Grandgirard, A.; Martine, L.; Joffre, C.; Juaneda, P.; Berdeaux, O. *J. Chromatogr. A* **2004**,

- 1040, 239-250.
109. Brown, A. J.; Jessup, W. *Atherosclerosis* **1999**, *142*, 1-28.
 110. Sottero, B.; Gamba, P.; Gargiulo, S.; Leonarduzzi, G.; Poli, G. *Curr. Med. Chem.* **2009**, *16*, 685-705.
 111. Lordan, S.; Mackrill, J. J.; O'Brien, N. M. *J. Nutr. Biochem.* **2009**, *20*, 321-336.
 112. Tai, C. Y.; Chen, Y. C.; Chen, B. H. *J. Food Drug Anal.* **1999**, *7*, 243-257.
 113. Chamberlin, E. M.; Tristram, E.; Utne, T.; Chemerda, J. M. *J. Am. Chem. Soc.* **1957**, *79*, 456-459.
 114. Terasawa, T.; Hirano, Y.; Fujimoto, Y.; Ikekawa, N. *J. Chem. Soc. Chem. Comm.* **1983**, 1180-1182.
 115. Foley, D. A.; O'Callaghan, Y.; O'Brien, N. M.; McCarthy, F. O.; Maguire, A. R. *J. Agr. Food. Chem.* **2010**, *58*, 1165-1173.
 116. McCarthy, F. O.; Chopra, J.; Ford, A.; Hogan, S. A.; Kerry, J. P.; O'Brien, N. M.; Ryan, E.; Maguire, A. R. *Org. Biomol. Chem.* **2005**, *3*, 3059-3065.
 117. Maguire, L.; Konoplyannikov, M.; Ford, A.; Maguire, A. R.; O'Brien, N. M. *Brit. J. Nutr* **2003**, *90*, 767-775.
 118. Adcox, C.; Boyd, L.; Oehrl, L.; Allen, J.; Fenner, G. *J. Agr. Food. Chem.* **2001**, *49*, 2090-2095.
 119. O'Callaghan, Y. C.; Foley, D. A.; O'Connell, N. M.; McCarthy, F. O.; Maguire, A. R.; O'Brien, N. M. *J. Agr. Food. Chem.* **2010**, *58*, 10793-10798.
 120. Koschutnig, K.; Heikkinen, S.; Kemmo, S.; Lampi, A. M.; Piironen, V.; Wagner, K. H. *Toxicol. in Vitro* **2009**, *23*, 755-762.
 121. Leonarduzzi, G.; Sottero, B.; Poli, G. *J. Nutr. Biochem.* **2002**, *13*, 700-710.
 122. Ryan, E.; Chopra, J.; McCarthy, F.; Maguire, A. R.; O'Brien, N. M. *Brit. J. Nutr* **2005**, *94*, 443-451.
 123. Herzog, H. L.; Payne, C. C.; Hershberg, E. B. *J. Am. Chem. Soc.* **1955**, *77*, 5324-5327.
 124. Chung, S. K.; Ryoo, C. H.; Yang, H. W.; Shim, J. Y.; Kang, M. G.; Lee, K. W.; Kang, H. I. *Tetrahedron* **1998**, *54*, 15899-15914.
 125. Beuchet, P.; Dherbomez, M.; Elkiel, L.; Charles, G.; Letourneux, Y. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1599-1600.
 126. Lorente, S. O.; Jimenez, C. J.; Gros, L.; Yardley, V.; Luca-Fradley, K.; Croft, S. L.; Urbina, J. A.; Ruiz-Perez, L. M.; Pacanowska, D. G.; Gilbert, I. H. *Bioorg. Med. Chem.* **2005**, *13*, 5435-5453.
 127. James, S. P.; Smith, F.; Stacey, M.; Webb, M. *J. Chem. Soc.* **1946**, 665-670.
 128. Dodgson, D. P.; Haworth, R. D. *J. Chem. Soc.* **1952**, 67-71.
 129. Fetizon, M.; Kakis, F. J.; IGNATIAD.V *J. Org. Chem.* **1974**, *39*, 1959-1962.
 130. Kim, S.; Oh, C. H.; Ko, J. S.; Ahn, K. H.; Kim, Y. J. *J. Org. Chem.* **1985**, *50*, 1927-1932.
 131. Koziara, A.; Zwierzak, A. *Tetrahedron Lett.* **1987**, *28*, 6513-6516.
 132. Campa, D.; Gioia, A.; Tomei, A.; Poli, P.; Barale, R. *Clin. Pharmacol. Ther.* **2008**, *83*, 559-566.
 133. Gates, M.; Tschudi, G. *J. Am. Chem. Soc.* **1952**, *74*, 1109-1110.
 134. Gates, M.; Tschudi, G. *J. Am. Chem. Soc.* **1956**, *78*, 1380-1393.
 135. Rice, K. C. *J. Org. Chem.* **1980**, *45*, 3135-3137.
 136. Evans, D. A.; Mitch, C. H. *Tetrahedron Lett.* **1982**, *23*, 285-288.
 137. Toth, J. E.; Hamann, P. R.; Fuchs, P. L. *J. Org. Chem.* **1988**, *53*, 4694-4708.
 138. Parker, K. A.; Fokas, D. *J. Am. Chem. Soc.* **1992**, *114*, 9688-9689.
 139. Hong, C. Y.; Kado, N.; Overman, L. E. *J. Am. Chem. Soc.* **1993**, *115*, 11028-11029.
 140. White, J. D.; Hrniciar, P.; Stappenbeck, F. *J. Org. Chem.* **1999**, *64*, 7871-7884.
 141. Taber, D. F.; Neubert, T. D.; Rheingold, A. L. *J. Am. Chem. Soc.* **2002**, *124*, 12416-12417.
 142. Stork, G.; Yamashita, A.; Adams, J.; Schulte, G. R.; Chesworth, R.; Miyazaki, Y.; Farmer, J. J. *J. Am. Chem. Soc.* **2009**, *131*, 11402-11406.
 143. Fernandes, L. C.; Kilicarslan, T.; Kaplan, H. L.; Tyndale, R. F.; Sellers, E. M.; Romach, M. K. *J. Clin. Psychopharm.* **2002**, *22*, 326-329.

144. Pianese, C. P.; Hidalgo, L. O. V.; Gonzalez, R. H.; Madrid, C. E.; Ponce, J. E. C.; Ramirez, A. M.; Moran, L. M.; Arenas, J. E. P.; Rubio, A. T. Y. G.; Uribe, J. O.; Abiuso, J.; Hanuch, J.; Alegria, J.; Volpi, J.; Flaskamp, R.; Sanjuan, A. P.; Gomez, J. M. G.; Hernandez, J.; Pedraza, A.; Quijano, D.; Martinez, C.; Castaneda, J. R.; Guerra, S. J. C.; Valencia, G. F. *Otology & Neurotology* **2002**, 23, 357-363.
145. Citrome, L. *Therapeutics and Clinical Risk Management* **2013**, 9, 235-245.
146. Schmutz, J. Hunziker, F. Kunzle, F.M. (Bern, Switzerland), US3546226, **1970**.

Chapter Two

Results and Discussion



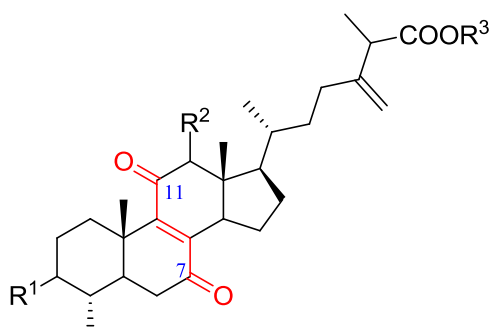
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2.1 Project Background

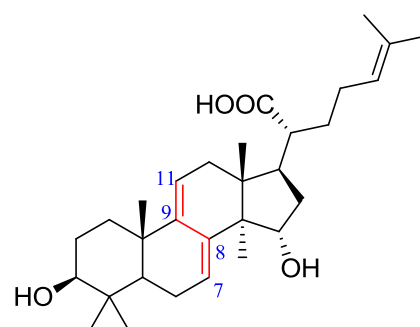
The concept for this project came from the reported anticancer activity of isolates from the medicinal mushroom *Antrodia camphorata*. From this rare mushroom, five lanostane and three ergostane type triterpenoids were isolated.¹ Certain isolates were shown to be cytotoxic against colon cancer and breast cancer cell lines, as discussed in the introductory chapter. A common thread of defining structural features is seen throughout these compounds. Specifically, the conjugated diketone at C-7 and C-11, the conjugated diene at C-7,8 – 9,11, and the alcohol group at C-3 (Figure 2.1).



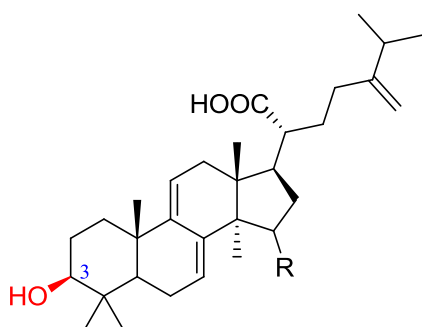
Isolate 1: R¹ = (=O), R² = H, R³ = CH₃

Isolate 5: R¹ = (=O), R² = R³ = H

Isolate 7: R¹ = R² = β-OH, R³ = H



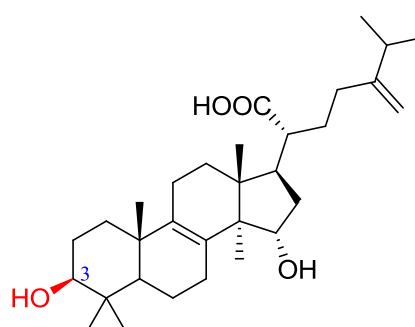
Isolate 4



Isolate 2: R = H

Isolate 3: R = α-OAc

Isolate 6: R = α-OH



Isolate 8

Figure 2.1

Therefore, we wished to exploit the structural similarities between these isolates and the commercially available lanosterol precursor, which contains the core A, B, C, D ring system in addition to the C-8,9 alkene, which would open up a range of core functionalisations and derivitisations, and the C-24,25 alkene which is susceptible to oxidations, epoxidations etc.

Recent work within our research group focussed on the synthesis of lanostane type natural products similar to the isolates from *Antrodia camphorata*.^{2,3} This previous work had concentrated on mimicking the structural motifs seen in these isolates, such as the conjugated diketone, the conjugated diene and the alcohol at C-3, as highlighted in Figure 2.1. In particular, the research investigated the derivitisation of lanostane steroids by modifying the ring structures to include these structural features. Through a variety of chemical transformations, such as allylic oxidations and epoxidations, a number of key targets were synthesised (Figure 2.2).

The work proved very successful, with five modified lanostane compounds being accepted for biological screening, (Figure 2.2). This evaluation was carried out by Professor John Luong of the Biotechnology Research institute, Quebec, Canada. The compounds were tested against *Spodoptera frugiperda* Sf9 insect cells using a technique called electric cell-substrate impedance sensing (ECIS).^{2,4} In brief, the technique performs real time cell monitoring through impedance measurement of a small electrode to AC current flow. When the cells are inoculated onto this small gold electrode, they behave like dielectric particles due to the insulating properties of their membranes. Thus, impedance increases with increasing cell coverage of the electrode. As cell morphology is extremely sensitive to chemical stimulus, the change in impedance of the electrode, upon addition of chemical compounds, can provide information on the compounds inhibitory and cytotoxicity effects.

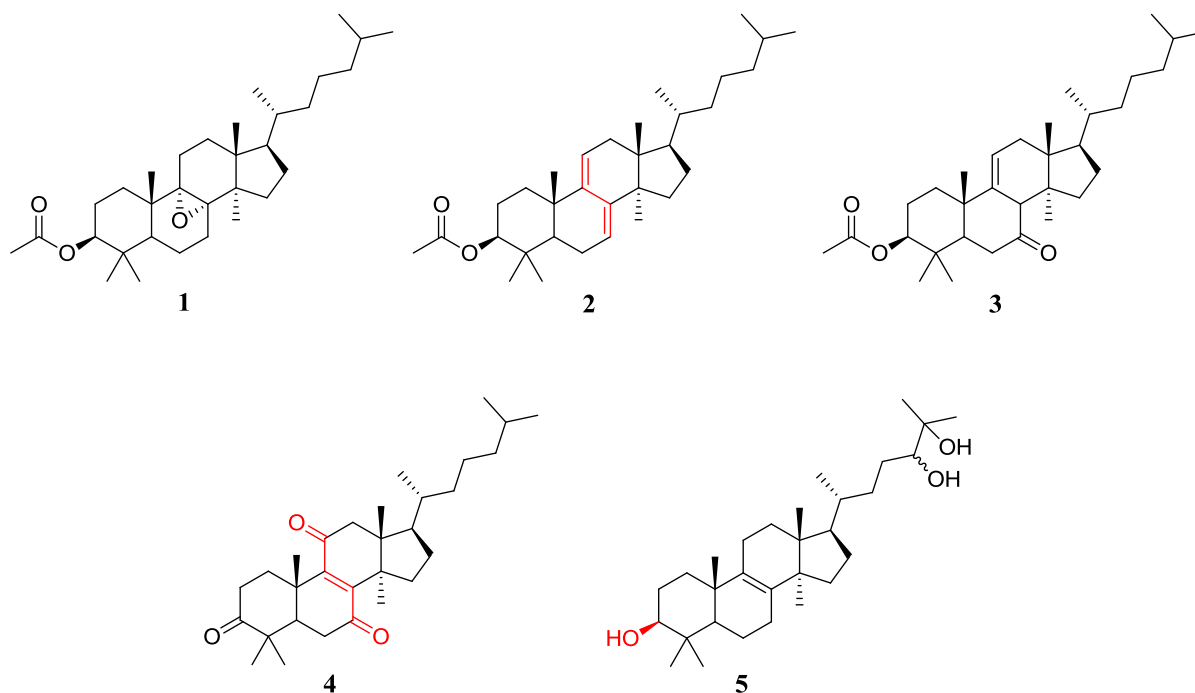


Figure 2.2

Compounds **1**, **2** and **3**, which have an ester group at C-3 and an aliphatic side chain in common, displayed no inhibitory activity against the Sf9 cells. Though the mode of action of steroids as transcription factors is well known,⁵ the specific mode of action of these lanosterol oxidation products as anticancer agents is not yet established; however it is possible that an alcohol group would lead to greater inhibition than an ester group due to its increased solubility, polarity and hydrogen bonding capabilities. The epoxide **1** is inactive up to a concentration of 150 μM , while both diene **2** and ketone **3** are inactive up to 300 μM and 100 μM respectively in this screening. However, these preliminary tests serve as an indication of possible activity only, and further screening of these compounds is necessary across a broad range of cell lines. By contrast, triketone **4** and triol **5** display more promising results against the Sf9 cells using the ECIS technique. After 20 hours the triketone displays an ECIS₅₀ (half inhibitory concentration using the ECIS method) of 150 μM . Characteristic features of this compound are the conjugated diketone moiety and the ketone at C-3, which may have increased its inhibitory properties. Triol **5** shows a lower inhibitory activity, with an ECIS₅₀ value of 250 μM after 20 hours. This compound was the only one to be tested in this screen with a functionalised side chain, and this too may have increased its inhibitory properties through increased solubility and polarity.

From the graph (Figure 2.3), it can be seen that the triol maintains a relatively constant ECIS₅₀ value over the monitored time period. By contrast, the inhibitory properties of the triketone significantly improve over time.

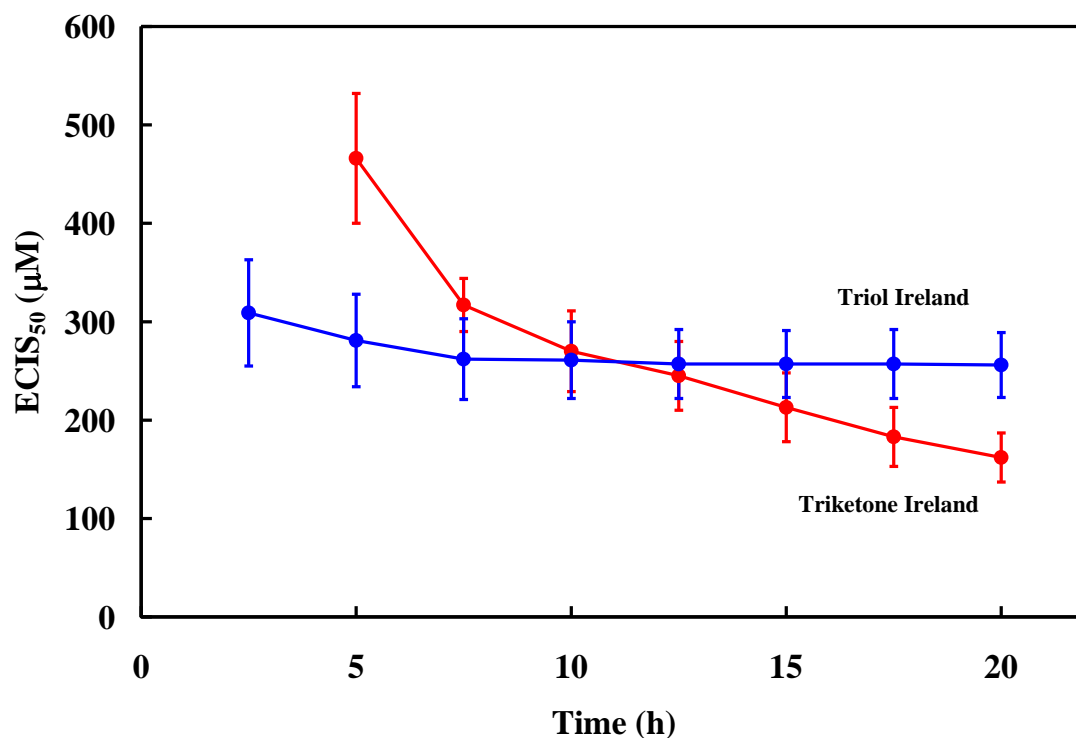


Figure 2.3

Initial stages of a collaboration between Professor John Luong and our research group culminated in a publication in 2010,² and we were keen to carry on this work, whilst moving the project in a new direction.

While these advances had been made in the derivitisation of the core four ring structure (Figure 2.2), the side chain, which has great potential for derivitisation, remained mostly unfunctionalised. The exploitation of this space was the basis for our continuation of this work.

2.2 Project Outline

Lanostane type natural products have been shown to display a wide range of biological effects, ranging from anti-tumour² to anti-fungal⁶ activity. The aim of the project was to synthesise structural analogues of these lanostane type natural products and evaluate their biological activity. These biological results would be used to strategically design the synthesis plans for future target molecules throughout the research. The project is subdivided into three main sections.

1. Lanosterol Oxidation Products (LOPs).

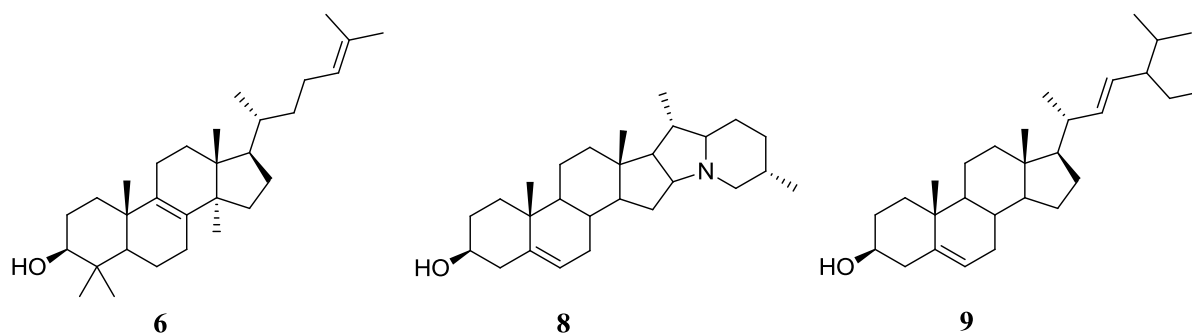
Initially our work focused on isolates from the medicinal mushroom *Antrodia camphorata*.³ Structural analogues of these isolates were synthetically accessible from our chosen precursor lanosterol **6**. A number of protection, oxidation, epoxidation and deprotection reactions constitute this section. Though lanosterol **6** is an excellent starting material for the synthesis of these analogues, the necessity to remove the dihydrolanosterol **7** analogue, which is present in commercial lanosterol, remained a key challenge during this body of work.

2. Amine Derivatives of Lanosterol.

Our attention turned towards the incorporation of tertiary nitrogen functionalities in the side chain of lanosterol **6**, by utilising the activity of the C-24,25 alkene. As well as having literature precedent for the synthesis of novel analogues of this type,⁶ collaborative work was carried out within the research group to mimic the chemistry and biological activity of the aglycone solanidine **8**. Reductive amination reactions emerged as the means of choice for the synthesis of these derivatives *via* a route devised in the LOPs section.

3. Cholestane Derivates of Biologically Active Amines.

The final section of the project focused on establishing a food related aspect to our research, as part of an ongoing collaboration with the food research group of Professor Nora O'Brien in UCC. We were attracted to working with stigmasterol **9** because it is a phytosterol present in the human diet,⁷ with a chemical structure suitable for modification by our established synthetic routes. The amine side chains showing the most promising biological activity from the previous lanostane section would be incorporated in the cholestane series derived from stigmasterol **9**.

Figure 2.4 Lanosterol **6**, Solanidine **8** and Stigmasterol **9**

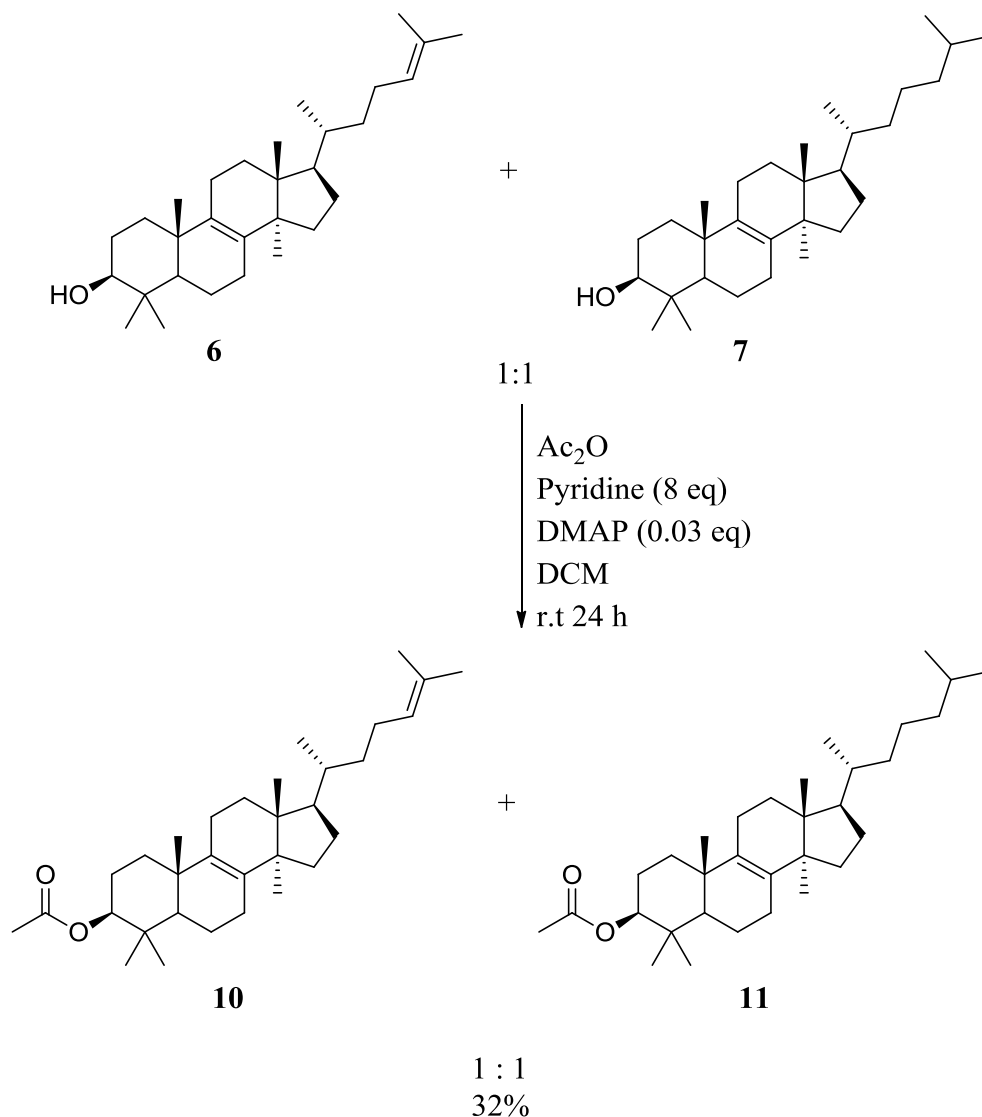
2.3 Lanosterol Oxidation Products (LOPs)

2.3.1 Purification of commercial lanosterol

In order to synthesise a new range of lanosterol oxidation products incorporating the structural features of compounds isolated from *A. camphorata*, lanosterol **6** was the most appropriate starting material. Lanosterol is also the direct precursor in the biosynthesis of lanostane type triterpenoids and is relatively inexpensive and readily available from a number of chemical suppliers. However, the purity of this lanosterol **6** is inadequate, as it contains approximately 50% dihydrolanosterol **7**, and other steroids, such as agnosterol, as trace impurities. Therefore, the separation of dihydrolanosterol from the commercial mixture to yield two viable starting materials was necessary. This separation was carried out as described by Kavtaradze *et al.*⁸

The initial step of the separation route involved the protection of the C-3 secondary alcohol of the 1:1 mixture of lanosterol **6** and dihydrolanosterol **7** through the introduction of an acetyl protecting group (Scheme 2.1).⁸ The acetate ester was used for the protection of this alcohol as its stability properties suited the conditions of our separation route. This protecting group is easily introduced to mask the alcohol, and could be removed at a later stage. Standard reagents acetic anhydride, dimethylaminopyridine and pyridine were employed for the acetylation reaction. The resulting off white residue was easily recrystallised from acetone to afford lanosterol acetate **10** and dihydrolanosterol acetate **11** (1:1) in 32% yield. The effect of the acetyl functionality is clearly observed in the ¹H NMR spectrum of the product.⁹ 3-CH shifts from 3.23 ppm in the mixture of alcohols **6** and **7**, to 4.50 ppm in the mixture of

acetates **10** and **11**. Furthermore, the 3H singlet at 2.05 ppm in the ^1H NMR spectrum of **10** and **11** is consistent with the presence of the new methyl group of the acetate ester. The ^{13}C NMR spectrum of the recrystallised product showed a shift in the 3-CH signal from 78.7 ppm to 80.9 ppm, along with the emergence of a new signal at 171.0 ppm, owing to the carbonyl functionality in the new acetate group.

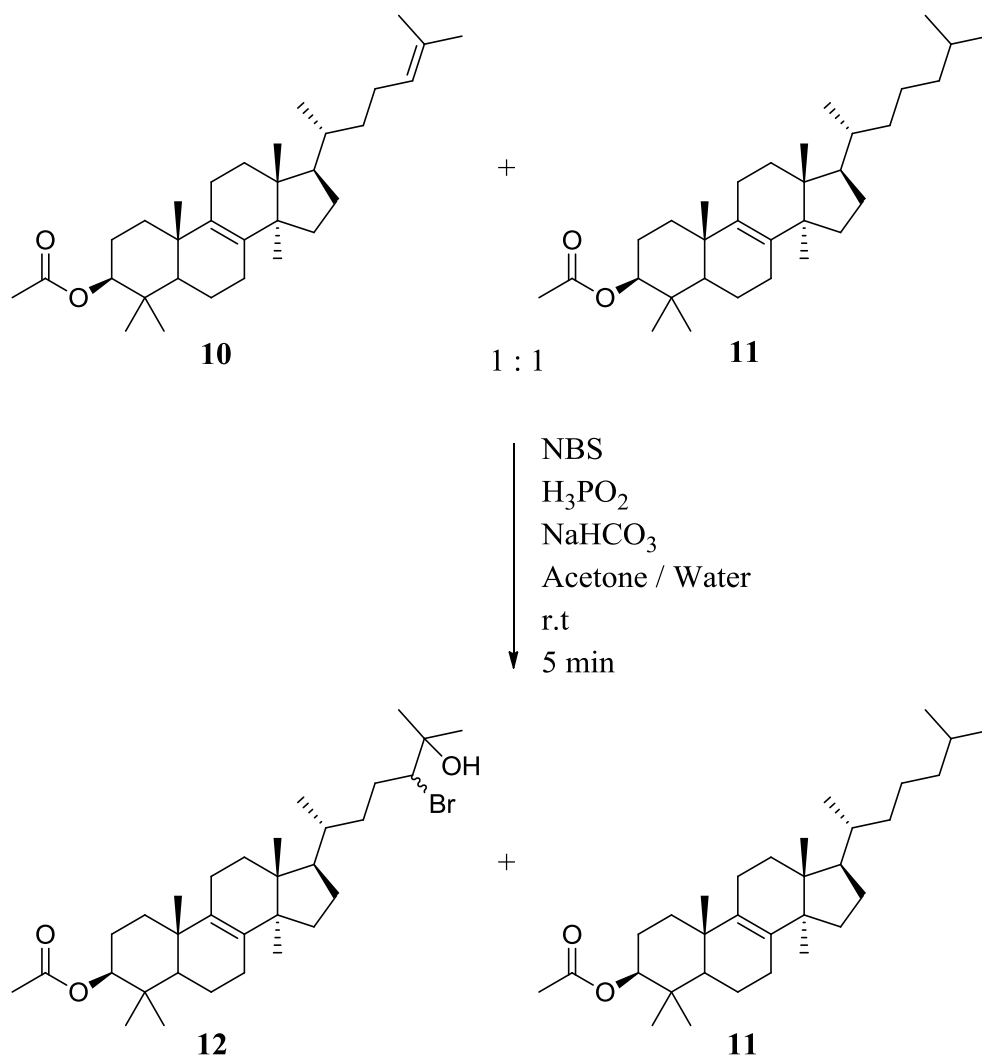


Scheme 2.1

Attempts to separate **10** and **11** by column chromatography at this stage were unsuccessful. Moreover, the attempted separation of trace steroid impurities from the bulk majority of **10** and **11** was also unsuccessful. Reports by Li¹⁰ and Williams¹¹ showed the separation of very closely related alkenes, such as *cis* and *trans* alkenes, using silica impregnated with silver

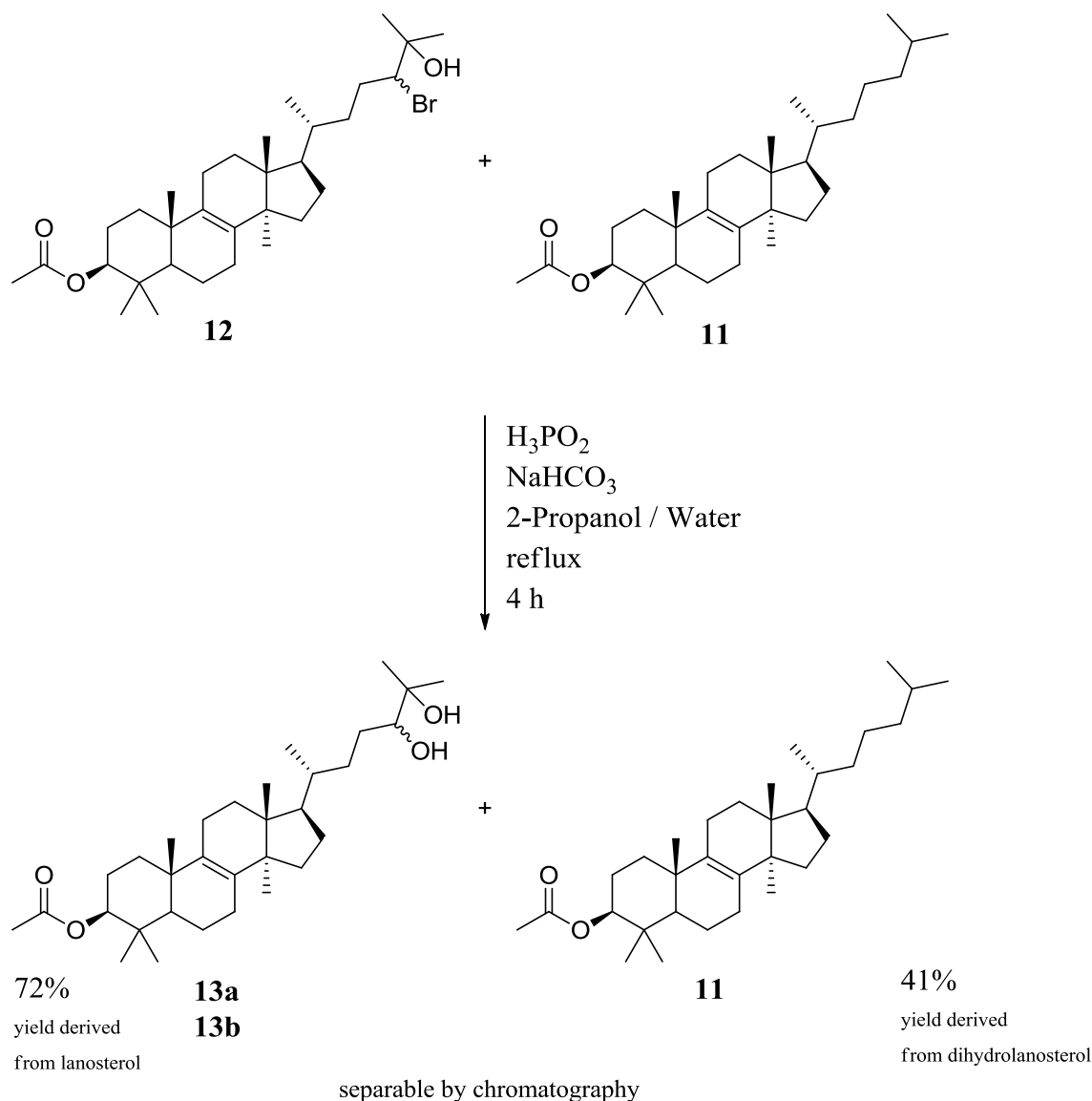
nitrate. This process involved treatment of the silica with 10% silver nitrate solution in methanol, before allowing the silica to dry overnight at $> 150\text{ }^{\circ}\text{C}$ in the dark. While **11** does not contain an alkene in its side chain, we were keen to attempt the chromatography described by Li and Williams as both **10** and **11** both contain an alkene at C-8,9. However, separation of **10** and **11** by chromatography using silica impregnated with silver nitrate also failed, although the literature indicated promising precedent for this separation.^{10,11} This shows that the dihydrolanosterol acetate **11**, in addition to trace impurities are too close in polarity to the desired lanosterol acetate **10** component of the mixture, and that side chain functionalisation is necessary in order to achieve a sufficient difference in polarity between the two compounds to allow for separation to take place by column chromatography.

The next step in the separation process is the hydroxy-bromination of the C-24,25 alkene of lanosterol acetate **10**. The mixture of acetates **10** and **11** was reacted with *N*-bromosuccinimide in aqueous acetone and hypophosphorous acid as a catalyst (Scheme 2.2). This reaction leaves the dihydrolanosterol acetate **11** component of the mixture unreacted, while the C-24,25 alkene of lanosterol acetate **10** reacts to form the halo-hydroxy intermediate **12**. The reaction proceeds regiospecifically, and affords only the 24-bromo, 25-hydroxyl moiety. This regiospecificity is attributable to the choice of solvent.¹² Mixtures of isomers are normally obtained; for instance, the same reaction in dichloromethane lacks this regiospecificity, and also yields the 24-OH, 25-Br compound. Upon addition of the *N*-bromosuccinimide, a colour change was observed from a clear to a bright yellow solution. The reaction was complete after 5 min, indicated by TLC analysis. As the bromo-hydroxy intermediate **12** decomposes readily,¹² the mixture of **11** and **12** was carried forward to the next reaction without further purification or analysis.



Scheme 2.2

The final step of the separation sequence involved refluxing the halo-hydroxy intermediate in 2-propanol and aqueous hypophosphorous acid for 4 h to form the target diol mixture **13a** and **13b**. Addition of sodium hydrogen carbonate generates the sodium hypophosphite salt *in situ*. 2-Propanol was the solvent of choice as competing nucleophilic reactions are avoided on the grounds of steric hinderance.¹³ The use of a different solvent, such as methanol or ethanol, may lead to the generation of undesired methyl or ethyl ethers in side reactions at the C-24 position. An acidic off white solid was obtained and this solid was washed with deionised water until it reached pH 7.



Scheme 2.3

When the side chain functionalisation of lanosterol acetate **10** was complete, there was now a sufficient difference in polarity between the diols **13a** and **13b** and the dihydrolanosterol acetate **11** to enable separation by column chromatography. Dichloromethane was used as the first eluant yielding **11** as a white solid in 41% yield as the first fraction from the column. When the eluant was changed to ethyl acetate, the diol mixture **13a** and **13b** was recovered as an off white solid in 72% yield as the second major fraction from the column. Some minor trace products with polarity ranging between **11** and **13a** and **13b** were distinguishable on the TLC plate of the crude material. These minor products were not further purified or characterised.

Diol **13** (Figure 2.5) was recognised by the doublet at 3.29 ppm corresponding to the CH of the 24*R* isomer and the singlet at 3.34 ppm corresponding to the CH of the 24*S* isomer in the ^1H NMR spectrum of the product.⁹ In the ^{13}C NMR spectrum the tertiary alcohol at C-25 appeared at 73.2 ppm, while a difference in the secondary alcohols at C-24 of **13a** and **13b** was evident by signals at 78.7 ppm and 79.6 ppm respectively. IR analysis showed a broad peak at 3431 cm^{-1} , consistent with the presence of the alcohol moieties in the mixture. The sharp signal at 1732 cm^{-1} is due to the carbonyl group of the acetyl functionality at C-3.

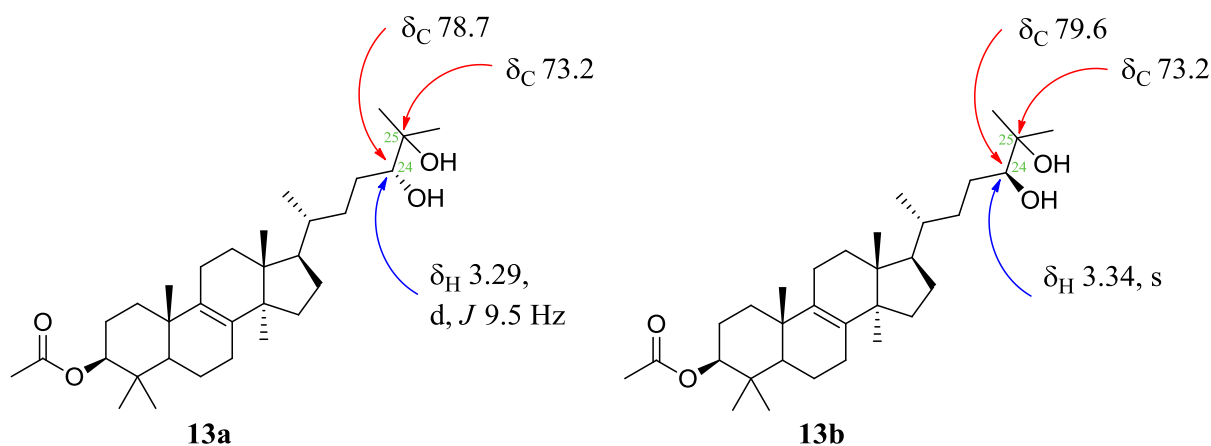


Figure 2.5

Following separation, both compounds served as viable starting materials for different aspects of the research. Diol **13** would serve as an invaluable starting material in the synthesis of target molecules with derivatised side chains. These were our main targets initially, as a number of the potent isolates from *Antrodia camphorata* have several oxygen containing functionalities in the side chains, including carboxylic acids and esters.³ In contrast, the dihydrolanosterol acetate **11** is unfunctionalised in the side chain. However, its usefulness cannot be devalued, as it would serve as a starting material in test reactions for synthetic modifications of the lanostane nucleus. This chemistry is equally as important in the research, as seven of the eight isolates from *Antrodia camphorata* possess either conjugated diketones at C-7,11, or conjugated dienes across the B and C ring motifs.³

Therefore, a process had been set in place, whereby test oxidations and reductions would be carried out on **11**, while diol **13** would be preserved for side chain chemistry where the lanostane nucleus modification routes had been established.

2.3.2 Target transformations

Following the synthesis of these two starting materials, we pinpointed the most interesting and useful synthetic transformations to target. The selected transformations (Figure 2.6), which would lead to an array of novel compounds, were devised in conjunction with the aforementioned interesting structural features of compounds isolated from *A. camphorata*.

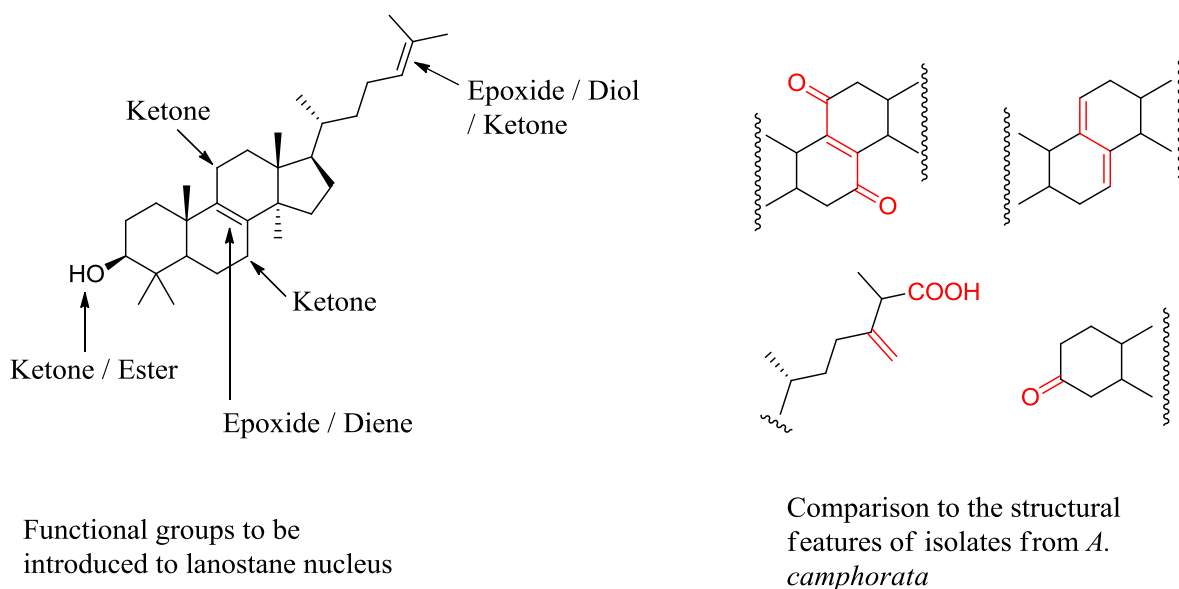


Figure 2.6

2.3.3 Allylic oxidations of C-8,9 alkene, and deprotections of C-3 alcohols

At this early stage in the project our aim was to expand on the previous research carried out and move this research in new directions, focussing on the lead compound **4**. Triketone **4**, the most biologically active compound synthesised in previous work within the research group, was used as the template for prospective target molecules. We wished to focus on the synthesis of novel compounds which would retain the C-7,11 conjugated diketone moiety, while progressing our work to incorporate side chain functionality. And so, an initial target molecule emerged (Figure 2.7), along with scope for the synthesis of derivatives.

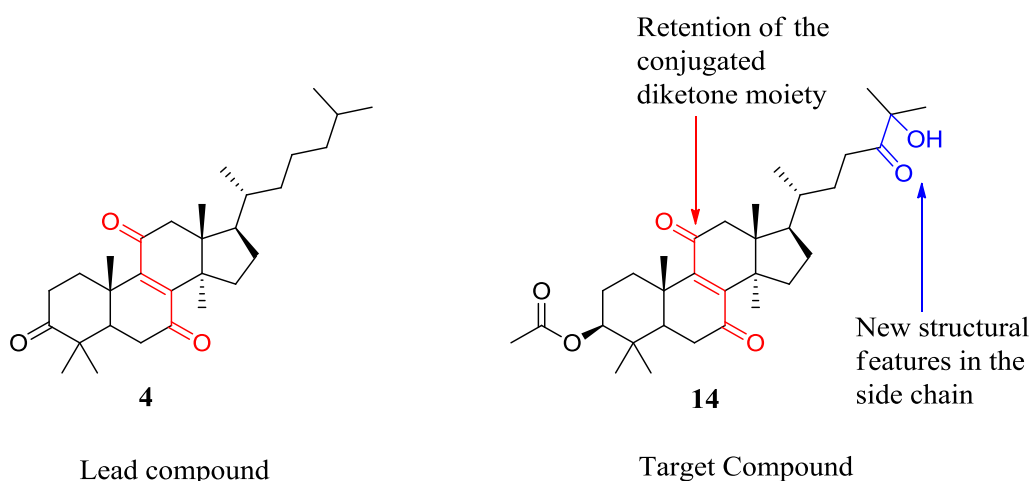
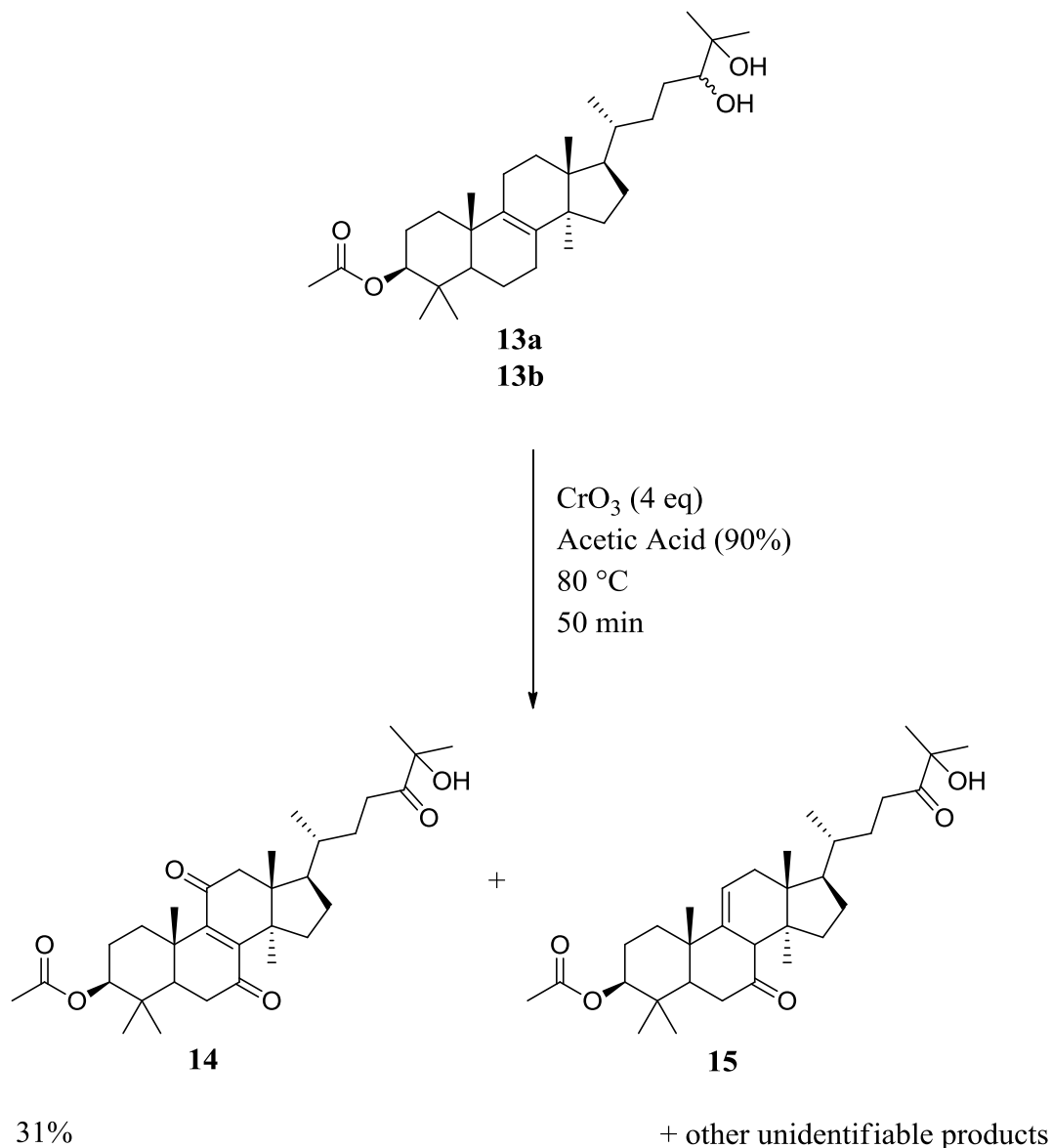


Figure 2.7

Previous research within our group successfully carried out the allylic oxidation of the C-7 and C-11 positions in the lanostane systems.² The oxidising agent chromium trioxide had been used for this transformation. We opted to continue with the CrO_3 method as it had shown the most success in the group to date. However, alternative methods of allylic oxidation would be explored at a later stage in this project. With our diol starting material, it was envisaged that the C-24 OH group would also be oxidised to the C-24 ketone during the course of this reaction, which would be very advantageous. Therefore, our first objective was the synthesis of our novel target molecule *via* the allylic oxidation of diol mixture **13a** and **13b** using chromium trioxide. Diol mixture **13a** and **13b** was reacted with 4 molar equivalents

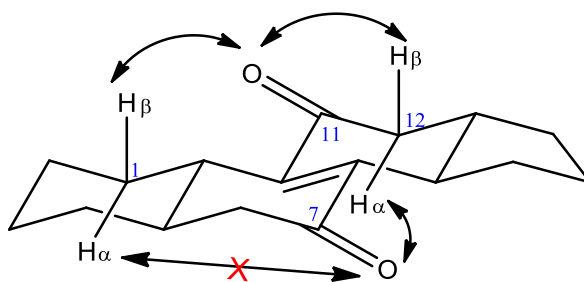
of chromium trioxide in refluxing acetic acid. Upon reaction initiation, the instantaneous colour change of the chromium trioxide from dark orange to dark green confirmed oxidation was taking place. As the substrate is being oxidised, the oxidising agent is itself being reduced, specifically from Cr^{VI} to Cr^{III} . The colour change of the chromium is consistent with its reduction, as chromium is known to be orange in its +6 oxidation state, and green in its +3 oxidation state. The oxidation reaction led to the formation of one major product **14**, plus a complex mixture of other products, one of which is identifiable as **15** (Scheme 2.4).



Scheme 2.4

The products were separable by column chromatography using hexane : ethyl acetate (80:20). The desired product **14** was isolated as a yellow solid in 31% yield as the second fraction from the column. As expected, the C-8,9 alkene underwent allylic oxidation to form the C-7,11 conjugated diketone. However, the new feature of interest in this product, the keto-alcohol in the side chain, is of greater significance. It marks the commencement of a new stage in the project, following on from the previous work. The secondary alcohol at C-24 in the diol starting material had been oxidised to a ketone, while the tertiary alcohol, which is not susceptible to oxidation, remained intact.

The product **14** showed characteristic new peaks in the 2.10 – 3.00 ppm region of the ^1H NMR spectrum. The ^1H signals at 2.60 and 2.78 ppm are characteristic of the 12-CHs in the β and α positions respectively. The downfield shift of these signals is good evidence for the formation of the ketone moiety at C-11. The doublet of triplets at 2.89 ppm was assigned to $1\beta\text{-CH}$, owing to the fact that C-11 is in close proximity to $1\beta\text{-CH}$ due to the conformational arrangement of the B and C steroidal rings (Figure 2.8). The ^{13}C NMR spectrum showed new signals in the carbonyl region at 201.9 and 202.2 ppm corresponding to ketones at C-11 and C-7 respectively. As the carbonyl group of the acetate ester is at the standard chemical shift value of 170.9 ppm, the new signal at ~ 180 ppm is being tentatively assigned as the ketone at C-24. Although this is a very low chemical shift value for a ketone, it is assigned as C-24 $\text{C}=\text{O}$ given that it is the only signal remaining in the carbonyl region (Figure 2.9). Moreover, the signals corresponding to 24-C-OH in the starting diol **13** at 3.29 and 3.34 ppm in the ^1H NMR spectrum are absent from the product **14**.



Proximity of $1\text{-CH}\beta$ to $11\text{-C}=\text{O}$, and both H's at C-12 to both ketones, is seen more clearly when steroidal rings are drawn in perspective. $1\text{-CH}\alpha$ is too far removed from $7\text{-C}=\text{O}$ to be affected. Surrounding methyl groups, acetate ester and side chain are removed for clarity.

Figure 2.8

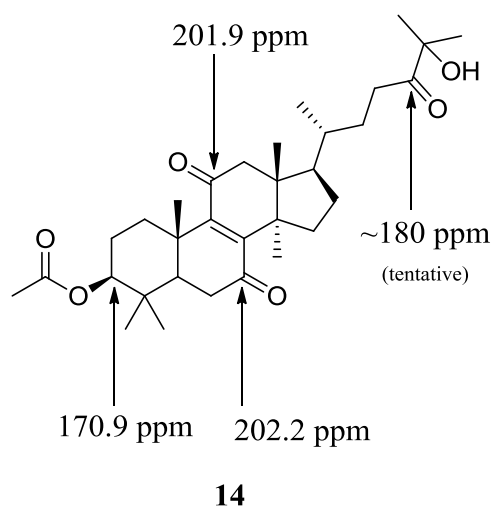


Figure 2.9 δ_C values for the noteworthy features of the novel compound **14**.

The side product **15** was isolated as part of a complex mixture as the first fraction from the column, and was an unexpected product of the reaction. It is being assigned as one compound in a mixture with trace amounts of a number of unidentifiable products. ^1H NMR spectroscopic evidence for this shift appears as a 1H singlet at 2.89 ppm which corresponds to $8\beta\text{-CH}$. Therefore the C-8,9 double bond has moved out of conjugation into the C-9,11 position. The 1H multiplet between 5.40 and 5.41 ppm represents the vinylic proton 11-CH, further verifying the existence of the C-9,11 double bond (Figure 2.10).

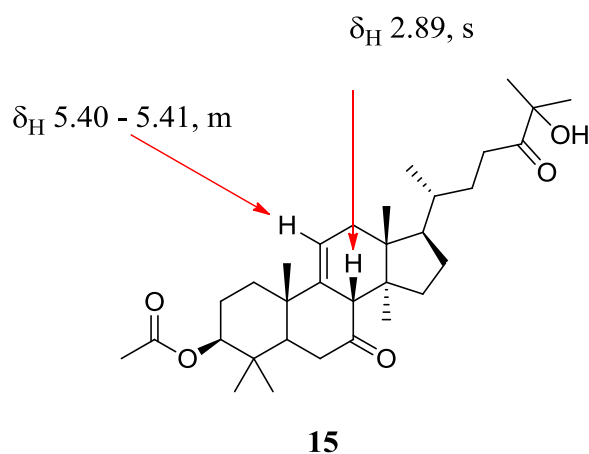


Figure 2.10 Side product **15**

Interestingly, in this product, the alkene bond shifts out of conjugation with the carbonyl group from C8-9 to C9-11. Usually conjugation is a favourable entity on electronic grounds, allowing free resonance over multiple bonds and increasing stability. However, in this case, one must consider the puckered nature of the C-C bonds in the chair conformation of cyclohexene. A plausible explanation for this uncharacteristic behaviour is that the conjugation may put too much strain on ring B, and thus the alkene bond moves out of conjugation to alleviate this strain (Figure 2.11).

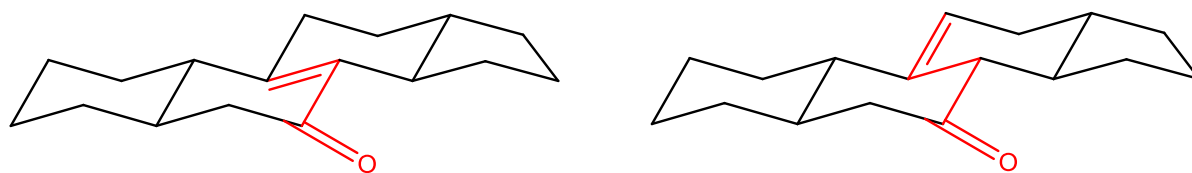
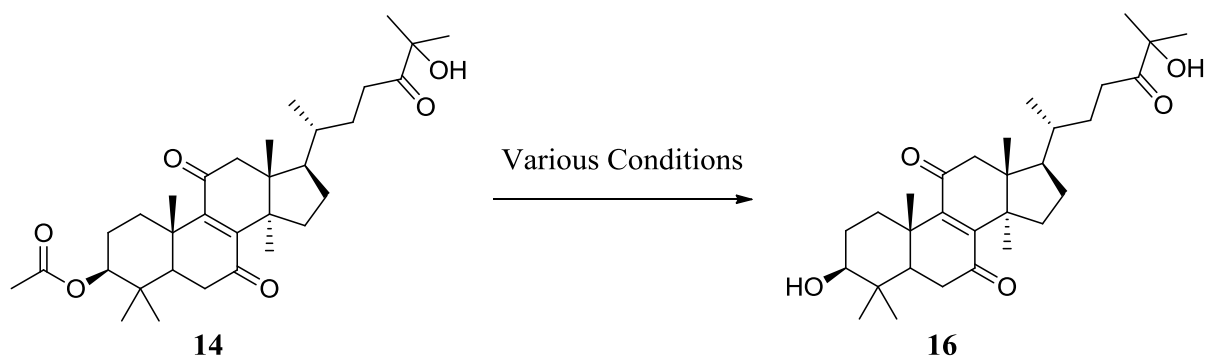


Figure 2.11

The new compounds synthesised, **14** and **15**, were intended to be evaluated for anti-cancer activity. Our main target, and major product of the reaction **14**, was sufficiently pure and stable to be evaluated for biological activity, the results from which will be presented in due course. However, given the impurity of side product **15**, it was not a viable candidate for this screening.

A significant number of the potent isolates from *Antrodia camphorata* contain a hydroxyl group at C-3. Therefore, after formation of the acetyl protected lanosterol derivative **14**, the next logical step was to proceed with deprotection of the alcohol at C-3 by removal of the acetate ester. This deprotection was investigated under various conditions using **14** as a substrate, and the results are shown in Table 2.1 below.

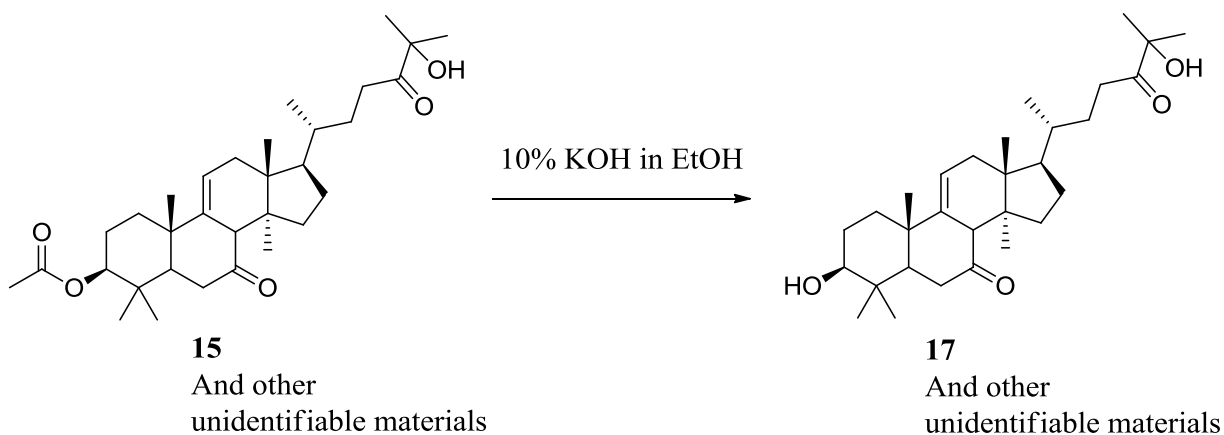
Table 2.1 Various conditions employed for the hydrolysis of **14**

Reagent	Equivalents	Solvent	Temperature	Time	Reaction Outcome
K ₂ CO ₃ (aq)	1.1	MeOH	r.t.	24 h	S.M.
K ₂ CO ₃ (aq)	2	MeOH	r.t.	24 h	S.M.
CH ₃ COCl	5	DCM/MeOH	r.t.	72 h	S.M.
KOH	10%	EtOH	60 °C	2 h	70%

The first set of conditions investigated were the use of 1.1 equivalents of aqueous potassium carbonate in methanol at room temperature for 24 hours.¹⁴ Spectroscopic analysis after this time showed that the identifiable peaks in the ¹H NMR spectrum corresponded to the starting material **14**. This material was retrieved in 100% recovery and the material was re-exposed to the base in the next test reaction. The concentration of potassium carbonate was increased to two equivalents and the reaction was repeated under the same conditions of temperature and time. ¹H NMR spectroscopic analysis revealed the same outcome that **14** was the main compound recovered, along with trace quantities of unidentifiable materials. This material was retrieved in 90% recovery. Previous work in the research group had employed acetyl chloride as a deacetylating agent.¹⁵ This procedure, using acetyl chloride in 1:1 anhydrous dichloromethane: methanol, was undertaken on the material from the failed potassium carbonate reactions. ¹H NMR analysis revealed a broad multiplet in the 3.20 – 3.50 ppm

region. This is the region where the signal of the 3-CH proton would be expected to appear. However, the desired splitting pattern of a doublet of doublets was not observed in this region. The successful deprotection was achieved with 10% potassium hydroxide in ethanol. The desired product **16** was formed in 70% yield and the characteristic disappearance of the ^1H doublet of doublets at 4.50 ppm and appearance of the signal at 3.20 ppm in the ^1H NMR spectrum was evidence of successful deprotection.

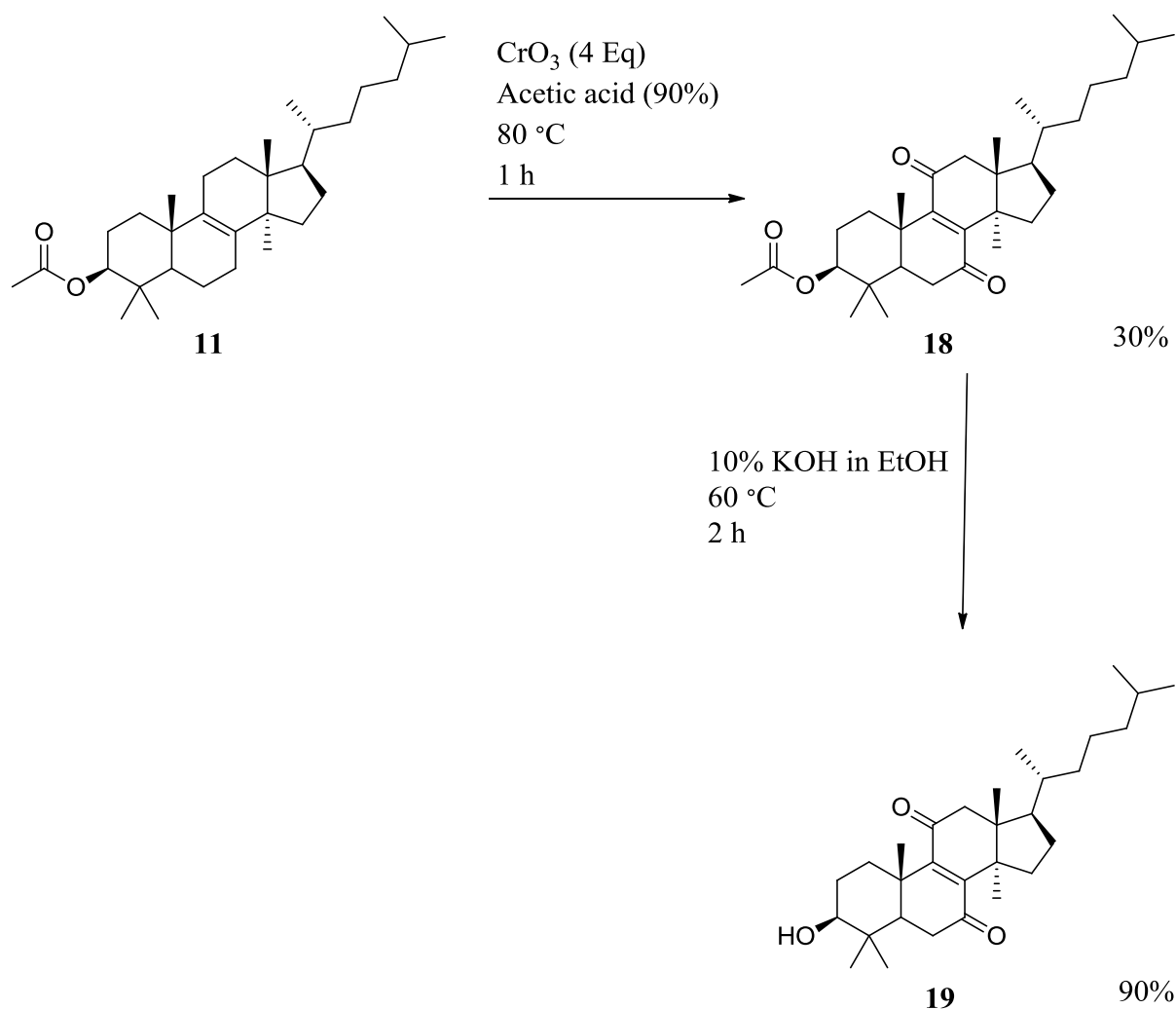
This deprotection was also carried out on **15**, which contained trace amounts of other unidentifiable products, with 10% potassium hydroxide in ethanol in an attempt to synthesise **17** (Scheme 2.5). ^1H NMR analysis of the crude material revealed a complex mixture of products. A shift in the 3-CH peak from 4.52 ppm to 3.30 ppm indicated successful alcohol deprotection. However other materials in the ^1H NMR spectrum of the crude material were unidentifiable due to the impure nature of the starting material. Purification of the product was not deemed viable due to limited quantities of material, and the relatively small amount of the desired product within the material, as seen by ^1H NMR analysis. The synthesis of **17** was not pursued further at this stage.



Scheme 2.5

After this initial allylic oxidation and deprotection had been investigated, our next target was to use dihydrolanosterol acetate **11** in a similar fashion. This would expand on our set of compounds with the conjugated diketone motif, and give a level of structural consistency in the first grouping of lanostane derivatives to be sent for biological evaluation.

Dihydrolanosterol acetate **11** also underwent allylic oxidation with 4 molar equivalents of chromium trioxide, to yield diketone **18** as a yellow solid. Deprotection of **18**, using the established successful method of KOH in EtOH gave **19** in 90% yield (Scheme 2.6).



Scheme 2.6

Characteristic signals in the ^1H NMR spectrum of the allylic oxidation product **18** at 2.61 ppm and 2.75 ppm represent $12\beta\text{-CH}$ and $12\alpha\text{-CH}$ respectively. The $1\beta\text{-CH}$ behaved in a similar fashion to the previous diketones synthesised, shifting downfield to 2.89 ppm due to its proximity to 11-C=O . The downfield shift of these signals indicates the proximity of the protons to the ketone functionalities at C-7 and C-11. The ^{13}C NMR spectrum is as expected,

with the characteristic C-7 and C-11 ketone signals appearing at 202.5 ppm and 202.0 ppm respectively (Figure 2.12). Other standard signals also appear in the ^{13}C NMR spectrum. The carbonyl group of the acetate ester appears at 170.8 ppm, and the C-8 and C-9 alkene signals remain at 150.7 ppm and 151.7 ppm respectively. This result is due to the high ratio of oxidising agent to substrate, and oxidation at both available allylic positions was achieved.

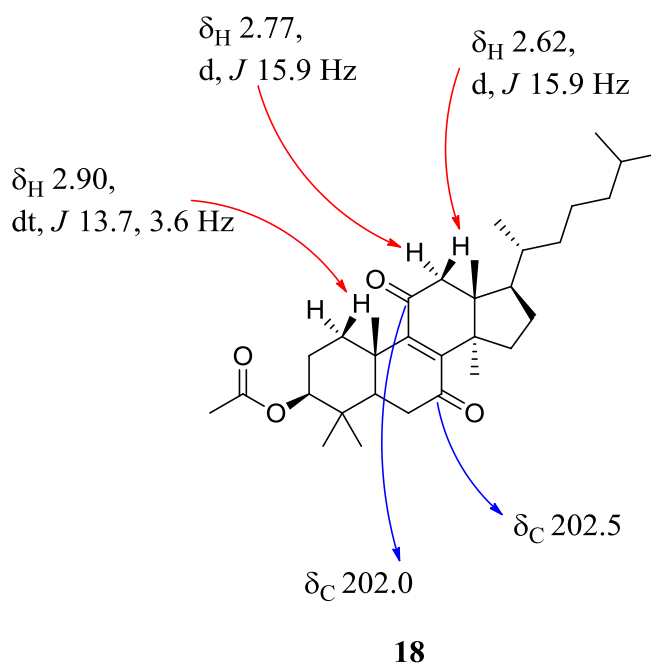


Figure 2.12 δ_{C} and δ_{H} values for the noteworthy features of **18**

The resulting diketone **18** was used for the next step which was deprotection of the C-3 alcohol group by removal of the acetate ester. This deprotection was accomplished successfully using the earlier optimised conditions of 10% KOH in EtOH yielding **19** (90%) following repeated chromatography. Again the characteristic disappearance of the ^1H doublet of doublets at 4.53 ppm and appearance of the new signal at 3.28 ppm in the ^1H NMR spectrum was evidence of successful deprotection (Figure 2.13).

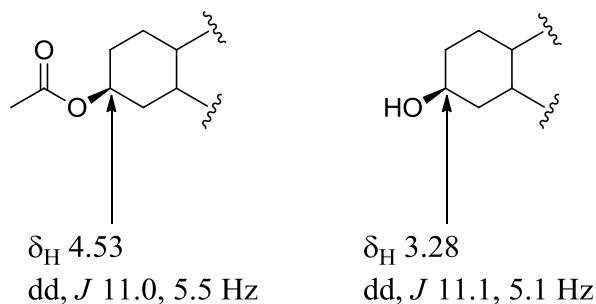
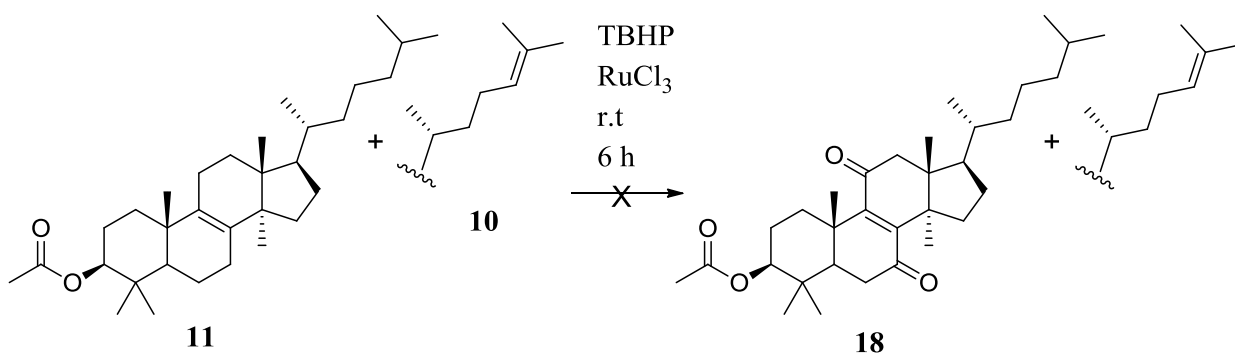


Figure 2.13

While the allylic oxidation using chromium trioxide is clearly an efficient method of synthesising ketones in these positions, it is not without its drawbacks. The Cr^{VI} reagent is highly toxic and carcinogenic, making it hazardous to researchers and the environment. Its disposal is not trivial, requiring separate containment and incineration of wastes. Therefore we had great interest in finding alternative methods for this oxidation. The dihydrolanosterol acetate **11** would be used as a substrate for these test conditions. This is an example of utilising **11** in order to test and optimise skeletal oxidations. Many literature reports detail unconventional allylic oxidation systems when the alkene bond is at the C-5,6 position.^{16,17} The introduction of a ketone moiety at C-7 appears to be considerably more difficult when the alkene is at the C-8,9 position. This may be due to the synergistic effects of increased steric hindrance exerted by additional methyl groups (ie. C-18, C-19 and C-30) around the site of reactivity, and also the steric unavailability of the tetrasubstituted C-8,9 double bond. Though previous work in our group attempted to oxidise the C-7 and C-11 positions using cumene hydroperoxide, t-butylhydroperoxide catalysed by both RuCl_3 and TBAB, and $\text{Rh}_2(\text{cap})_4$ and K_2CO_3 , these attempts had been unsuccessful and chromium reagents were instead employed.

The ruthenium trichloride – ^tbutyl hydroperoxide allylic oxidation of lanosterol derivatives is reported in the literature.¹⁸ A noteworthy property of ruthenium complexes is the existence of several oxidation states, most of which are kinetically inert. These oxidation states range from 0 to +8 and –2; the +2, +3 and +4 oxidation states being the most common. In the case of ruthenium chloride, ruthenium exists in its +3 oxidation state, one of its most stable states. ^tButyl hydroperoxide is a commonly used oxidising agent, most notably used in the Sharpless epoxidation. It is supplied as a 69 – 70% aqueous solution due to its instability at higher concentrations. This is the concentration of the oxidant used in the aforementioned literature example of the reaction.

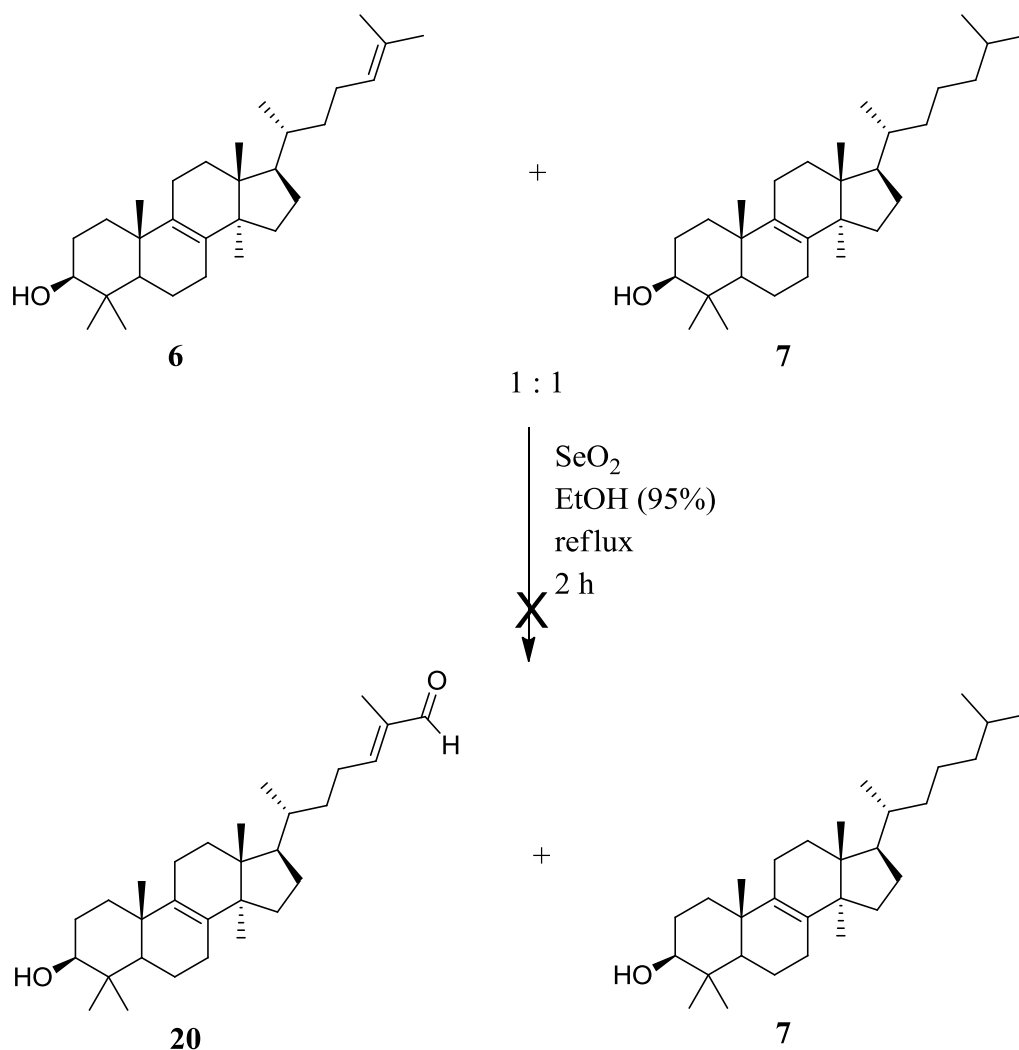
In this literature example, where the substrate was identical to our starting material **11**, the authors report an 89% yield of product **18**. However in our hands, using an inseparable mixture of **10** and **11** (Scheme 2.7), observation of the ^1H NMR spectrum of the crude material showed no evidence of formation of the desired product. It has been reported in the literature that ruthenium trichloride is very hygroscopic and absorbs some atmospheric moisture in the form of water of crystallisation, thus rendering its catalytic efficiency diminished.¹⁸ The reaction was repeated, doubling the concentration of the catalyst from 1 mol% to 2 mol%. However, no evidence of product formation was seen on this occasion. As the reaction was unsuccessful in our hands after repeated attempts, it was not further pursued using this reagent. Future work will involve investigations into methods of allylic oxidation without the use of chromium compounds, as this remains a desirable goal.



Scheme 2.7

Though the allylic oxidation of the C-8,9 alkene is difficult using milder reagents such as *t*-butyl hydroperoxide and ruthenium trichloride, the alkene in the side chain at C-24,25 is theoretically more susceptible to an allylic oxidation under mild conditions, given its exposed position. Therefore, we were also interested in allylic oxidation reactions which could be carried out on the lanosterol side chain.

Thus, an attempted allylic oxidation of the C-24,25 alkene was carried out on the inseparable mixture of lanosterol **6** and dihydrolanosterol **7** using selenium dioxide, in an attempt to exploit the alkene functionality present in the lanosterol side chain, and synthesise **20** (Scheme 2.8).



Scheme 2.8

This reaction was also unsuccessful in our hands, although Zhao reports the allylic oxidation of an isopropylidene group using selenium dioxide.¹⁹ A majority of starting material was recovered, with evidence of a trace amount (~6%) of product formation owing to the appearance of a broad singlet at 9.34 ppm in the ^1H NMR spectrum. Due to the limited amount of desired product within the crude material, purification was not attempted. This transformation could be revisited in future work, however, to date, chromium trioxide remains the best reagent for allylic oxidations in our work.

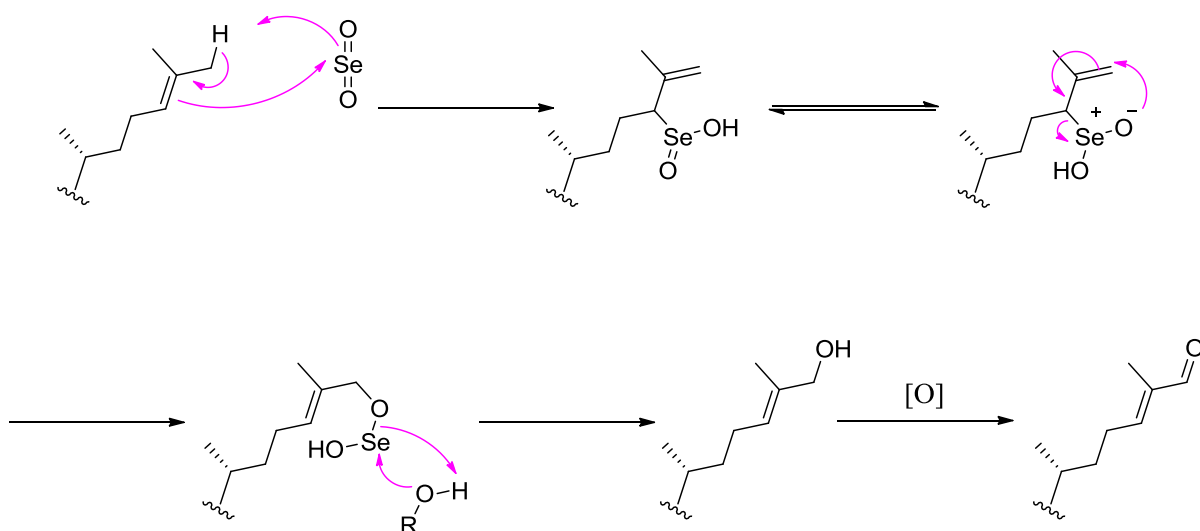


Figure 2.14 Mechanism of allylic oxidation using selenium dioxide

2.3.4 Revised route to new starting material; 3 β -acetoxy-5 α -lanost-8-ene-24-al 21

Though synthesis of the starting material diol **13** proved useful, both in order to separate the dihydrolanosterol component of the mixture, and as a precursor for side chain transformations, a C-24 aldehyde intermediate seemed to be a useful starting material for our work, due to the wide range of derivitisation prospects associated with an aldehyde group (Figure 2.15). Such derivitisations include Wittig reactions, Grignard reactions and reductive aminations, which will be discussed in due course.

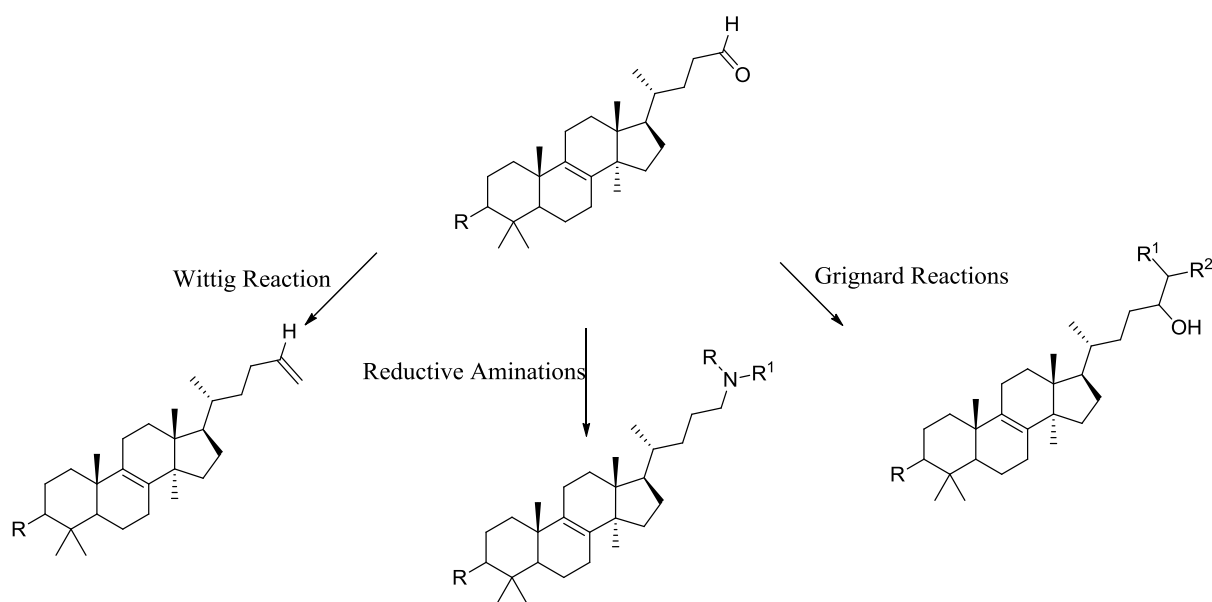
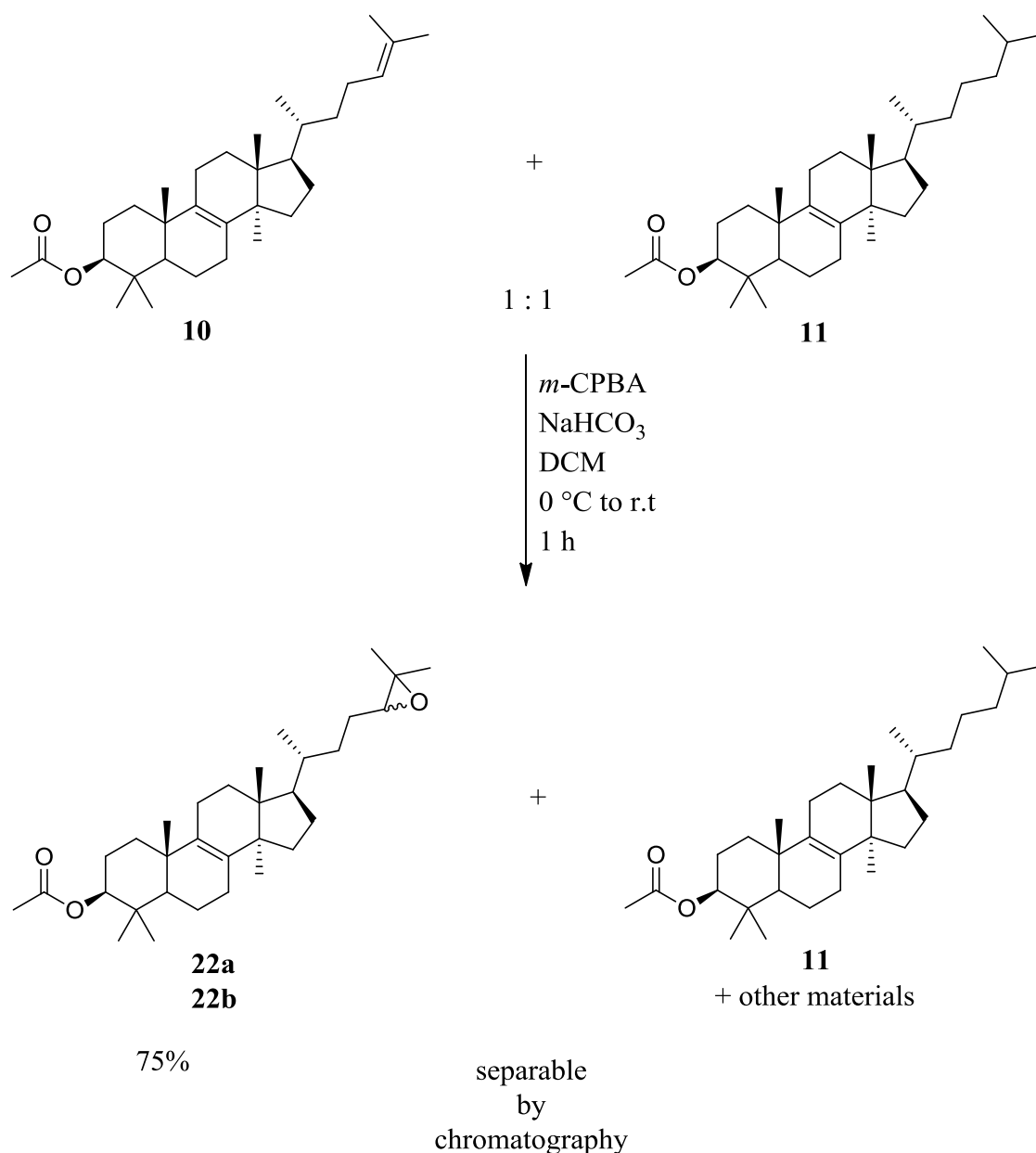


Figure 2.15

A synthetic route to this aldehyde, which proceeded through the selective epoxidation of C-24,25, was chosen. This route to the aldehyde starting material **21** had major advantages over the route to diol starting material **13**. Firstly the route involved two steps rather than three: the aldehyde was synthesised by formation of the side chain epoxide **22** (Scheme 2.9) followed by oxidative cleavage to yield **21** (Scheme 2.10), whereas the route to diol **13** involved the protection of the C-3 alcohol, followed by halohydrin formation, followed by diol formation. Secondly, there were less side products formed which in turn led to less tedious purification. This route led to aldehyde **21** which would become crucial in future work.



Scheme 2.9

The selective epoxidation of the lanosterol acetate component of the mixture of **10** and **11** proceeded in good yield (75%) under mild conditions and short reaction time. The C-24,25 alkene is epoxidised in preference to the C-8,9 alkene due to it being less sterically hindered. The epoxide **22** is formed as a 1:1 mixture of *R* and *S* isomers, and these isomers were distinguishable by signals in their ^{13}C NMR spectra.^{9,20}

The epoxide **22** is distinguished by the characteristic signals in the ^1H NMR spectrum. The triplet at 5.10 ppm corresponding to 24-CH of the starting alkene **10** had disappeared, and a new triplet at 2.69 ppm corresponding to 24-CH in the product **22** was observed. The absence of the C-24,25 alkene signals at 125.3 ppm and 130.9 ppm in the ^{13}C NMR spectrum of the starting material **10** and the presence of new epoxide signals at ~58 ppm and ~64 ppm in the product **22** is further evidence for this transformation. Peaks in the ^1H NMR spectrum are indistinguishable for the *R* and *S* isomers. However, clear differences can be deciphered in the ^{13}C NMR spectrum. Key distinctions between the *R* and *S* isomers are seen at C-12, C-16, C-17, C-20, C-22, C-23, C-24, C-25, and C-27.

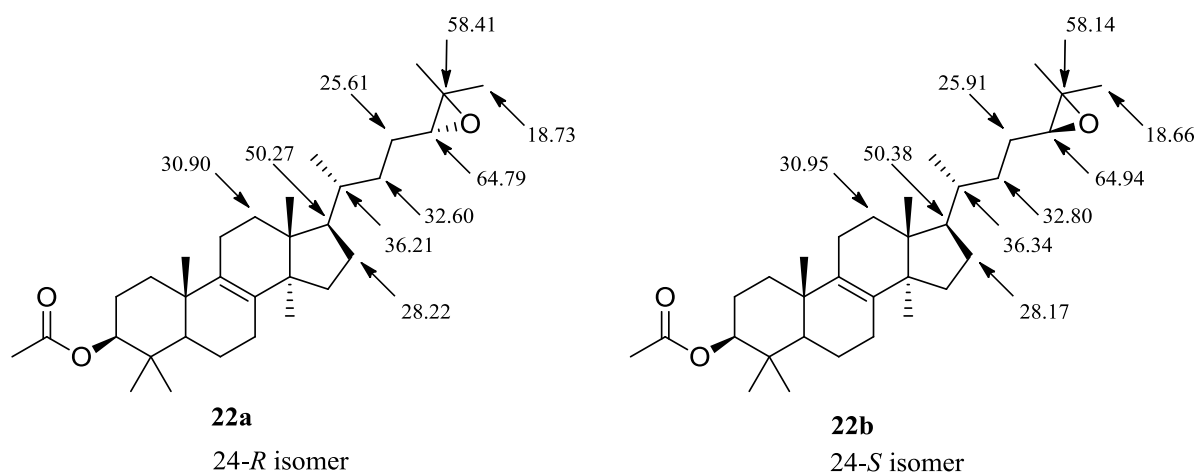
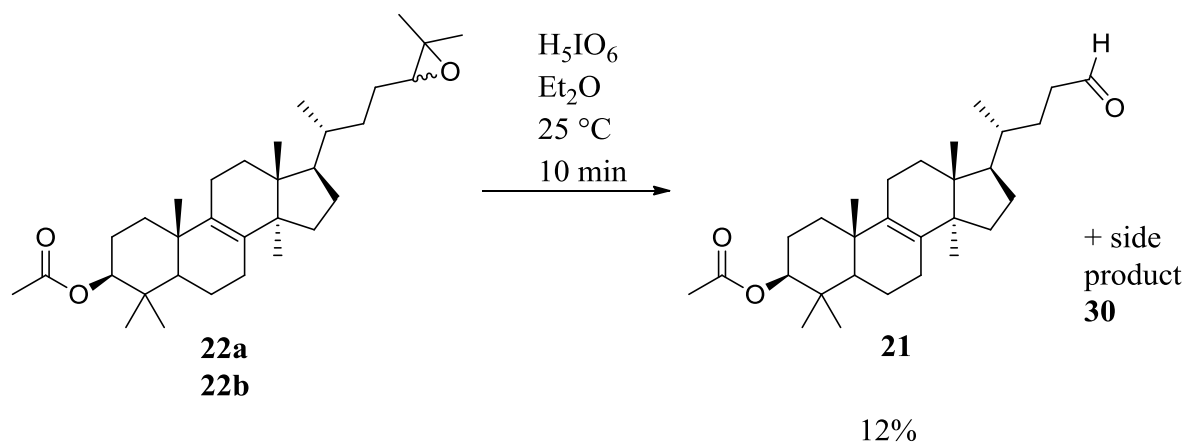


Figure 2.16

Table 2.2

Carbon	δ_C for 24- <i>R</i> isomer	δ_C for 24- <i>S</i> isomer
12	30.90	30.95
16	28.22	28.17
17	50.27	50.38
20	36.21	36.34
22	32.60	32.80
23	25.61	25.91
24	64.94	64.79
25	58.41	58.14
27	18.73	18.66

It was not necessary to attempt to separate these isomers, as our synthetic route involved the oxidative cleavage of the epoxide in the next step. This was achieved using periodic acid in diethyl ether under mild conditions with a very short reaction time of 10 minutes.²¹ It is worth noting the ease with which this cleavage took place, due to the steric availability of the epoxide at the end of the steroidal chain. The yield reported in Scheme 2.10 below is the yield of analytically pure product, with a further ~ 80% of the sample having a purity $\geq 95\%$. The formation of side product **30** will be discussed in due course.



Scheme 2.10

Mechanistically this reaction proceeds *via* initial acid ring opening of the epoxide (Figure 2.17). The periodic acid H_5IO_6 , which can be thought of as HIO_4 with two H_2O molecules attached, forms a five membered cyclic periodate intermediate with the diol. The concerted

collapse of this five membered intermediate leads to the formation of two carbonyl moieties; in our case, an aldehyde and a ketone. By fortunate coincidence, the ketone side product of this reaction is, in fact, acetone. Therefore, removal of the side product by column chromatography was not necessary, as it was lost in the concentration of the crude product under reduced pressure.

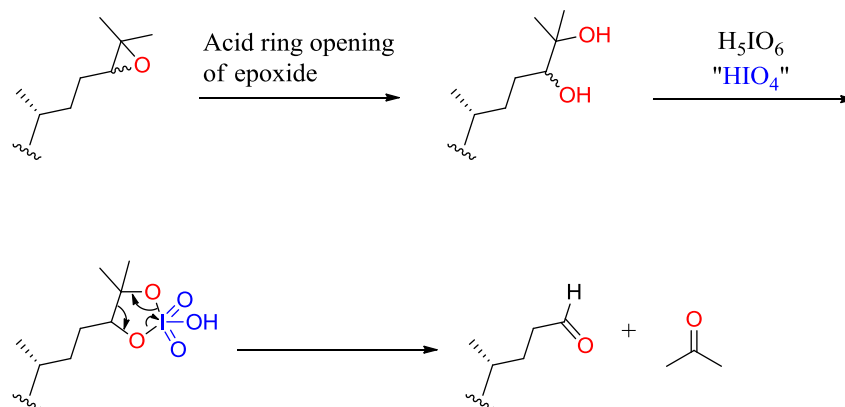


Figure 2.17

The formation of the aldehyde **21** was evident from a new triplet at 9.77 ppm in the ^1H NMR spectrum corresponding to 24-CH, and a new multiplet between 2.31 ppm and 2.52 ppm corresponding to 23-CH₂. The absence of the 24-CH epoxide signal at 2.69 ppm in the starting epoxide **22** was also observed upon analysis of the ^1H NMR spectrum. By comparison of the ^{13}C NMR spectra, the transformation is also evident. A new signal at 203.3 ppm in the ^{13}C NMR spectrum of the product is clear evidence for the formation of the desired aldehyde **21** (Figure 2.18).

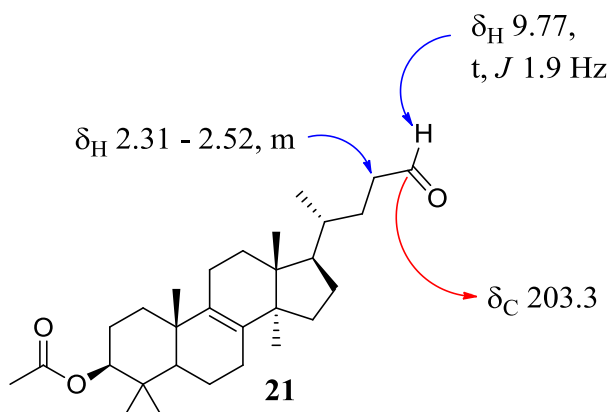


Figure 2.18

As already mentioned, the successful synthetic route to this aldehyde **21** would open up an exciting area of the project in the future.

2.3.5 Biological Testing 1: Lanosterol Oxidation Products

At this stage in the project we began our first biological evaluation of the products synthesised. The biological testing results would serve as a tool in strategically planning the next stage of synthetic chemistry. Compounds which showed good biological activity would be used as templates, and their functional groups would be incorporated as much as possible in the next batch of compounds. This first batch of lanosterol oxidation products was screened against two different cancer cell lines. The CaCo-2 cells are human colon cancer cells and the U937 cells are human lymphoma cells.²² The results are presented in terms of percentage viability of the cells after treatment with the test compounds in question. A low percentage viability relative to the concentration of compound used, indicates a positive result. For example, 5 – 10% viability of the cells using a concentration of 5 – 10 $\mu\text{g/mL}$ of test compound would be considered a good result. The evaluation of our first set of compounds is presented below (Table 2.3):

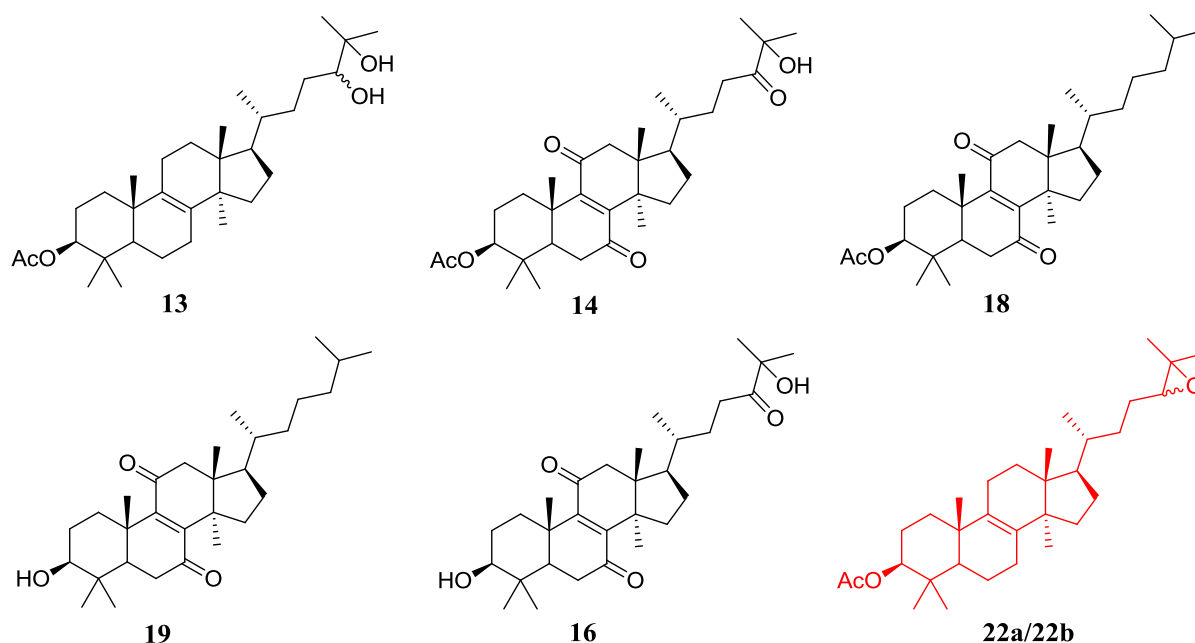


Figure 2.19

Table 2.3

	% Viability of CaCo-2 cells		% Viability of U937 cells	
	50 $\mu\text{g/ml}$	200 $\mu\text{g/ml}$	50 $\mu\text{g/ml}$	200 $\mu\text{g/ml}$
13		107.63 \pm 1.63		78.87 \pm 0.32
14		74.77 \pm 3.47		80.45 \pm 1.00
18		86.58 \pm 2.81		79.96 \pm 3.20
19		88.15 \pm 1.92		94.54 \pm 3.31
16		96.07 \pm 3.89		95.01 \pm 2.35
22	95.63 \pm 1.03		10.43 \pm 2.40	

% Viability of cells at
Compound Concentration of 200 $\mu\text{g/mL}$

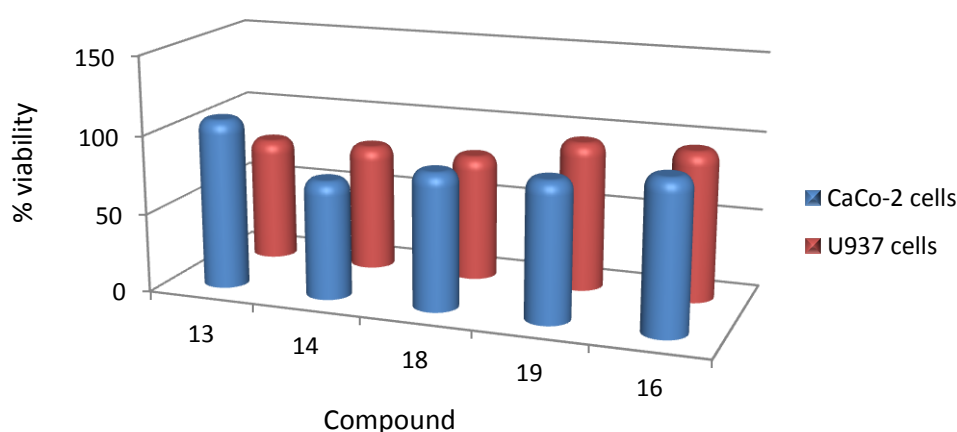


Figure 2.20: Percentage viability of CaCo-2 and U937 cells following treatment with 200 $\mu\text{g/mL}$ of compounds **13**, **14**, **18**, **19** and **16**.

It is evident that the epoxide **22** displayed the highest cytotoxicity, at a concentration of 50 $\mu\text{g/mL}$ giving ~ 10% viability. This result means that ~ 90% of U937 cells were killed when treated with 50 $\mu\text{g/mL}$ of epoxide **22**.

We were disappointed with the poor results from **14**, **18**, **19** and **16**, in these cell lines. As discussed earlier, the conjugated diketone was one of our biggest target structural features owing to its presence in many *A. camphorata* isolates. While these compounds did not show biological activity in the two cell lines tested, they will be screened against a larger number of cell lines in the future, as they contain interesting and highly oxygenated structural features.

Despite our disappointment with the poor activity of the diketones, we gained the valuable knowledge that the epoxide was the most active compound in this initial screen. Our aim was to take the most positive result from this screen and utilise the new biological knowledge as efficiently as possible. Our initial target was to incorporate epoxide functionality in further derivatives.

2.3.6 Synthesis of side chain epoxide derivatives 3-hydroxy-24*R*,25-epoxy-5 α -lanost-8-ene **23** and 3-keto-24*R*,25-epoxy-5 α -lanost-8-ene **24**

The next objective was to alter the functionality at carbon 3, while maintaining the epoxide incorporated into the side chain of the lanostane compound. Our next targets emerged as the alcohol **23** and ketone **24** at carbon 3 (Figure 2.21). Considering the structures of the biologically active isolates from *Antrodia camphorata* in conjunction with our first set of biological results, it appeared from our tests to date that the conjugated diketone was not as active as originally anticipated. The ketone at C-3, however, was still a viable target to pursue.

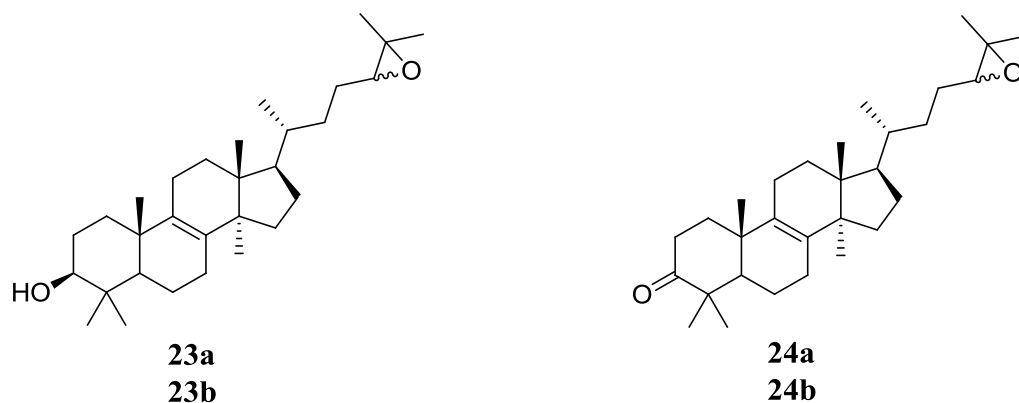
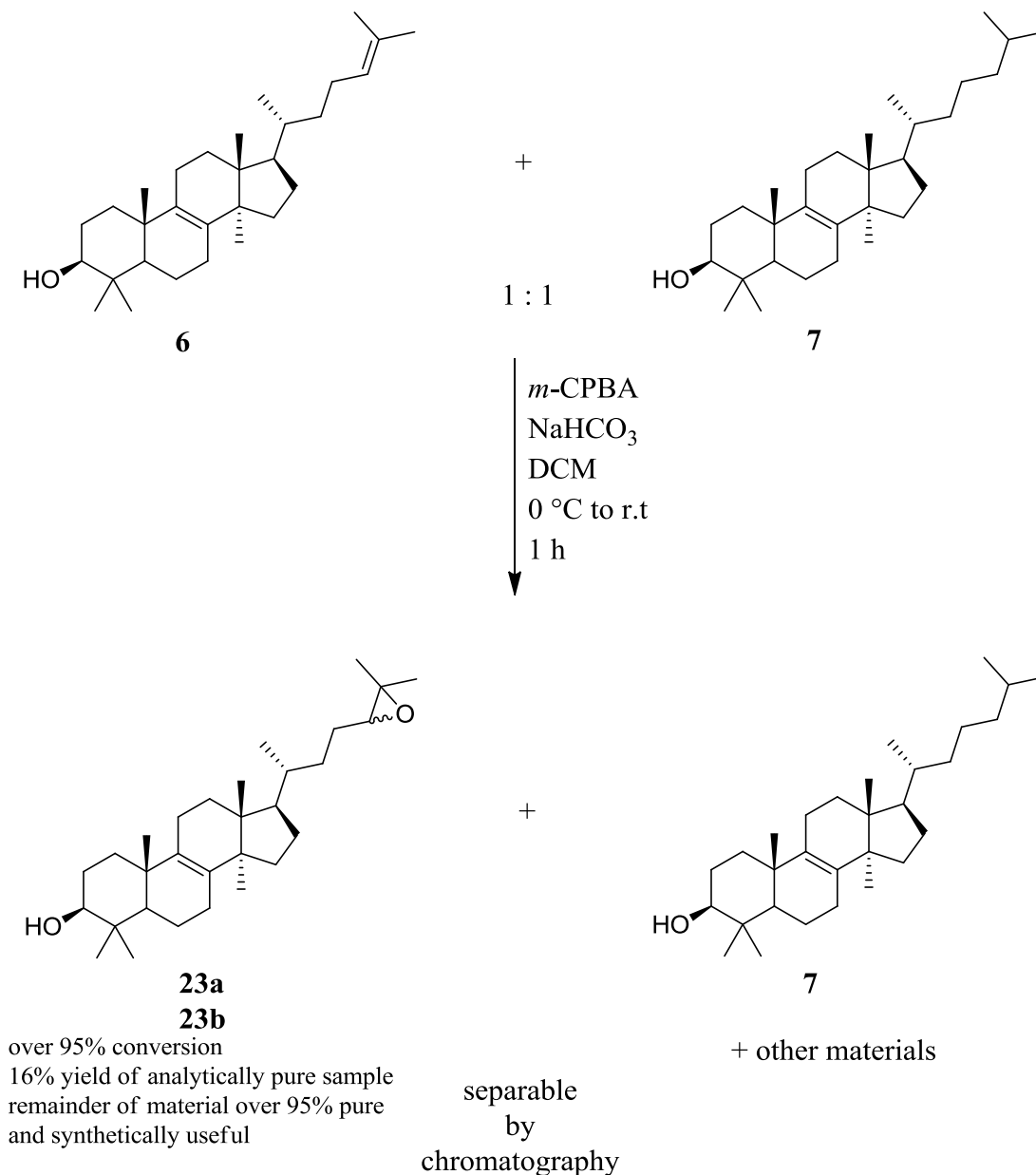


Figure 2.21

Firstly, the target epoxide with C-3 alcohol **23** was found to be relatively straightforward to synthesise. Given that the commercial lanosterol already has the alcohol in place at C-3, our synthetic strategy involved the selective epoxidation of the C-24,25 alkene and separation of the dihydrolanosterol species **7** (Scheme 2.11). The 16% yield of epoxides is the analytically

pure sample, the remainder of material is of a purity $\geq 95\%$, which is sufficiently pure for further synthetic work.

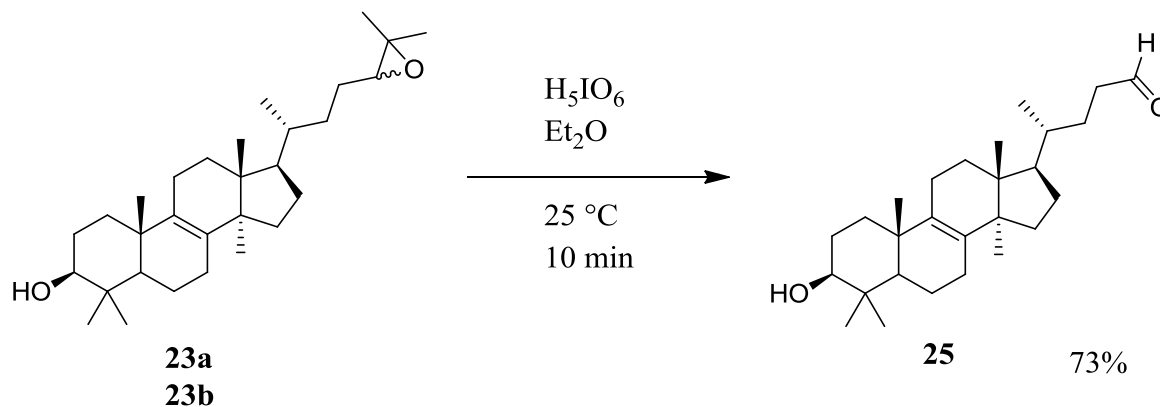


Scheme 2.11

The epoxide is again distinguished by the characteristic peaks in the ^1H NMR spectrum. The triplet at 5.10 ppm corresponding to 24-CH of the starting alkene **6** had disappeared, and a new triplet at 2.69 ppm corresponding to 24-CH in the product **23** was observed. The absence of the C-24,25 alkene signals at 125.2 ppm and 130.9 ppm in the ^{13}C NMR spectrum of the starting material **6** and the presence of new epoxide signals at ~58 ppm and ~64 ppm in the

product is supporting evidence for this transformation. This compound was synthesised in 57% yield and was available for the next round of biological screening.

It was logical at this stage to mimic the chemistry of the related C-3 acetate compound **22**, by performing an oxidative cleavage on epoxide **23**, to yield aldehyde **25** (Scheme 2.12).

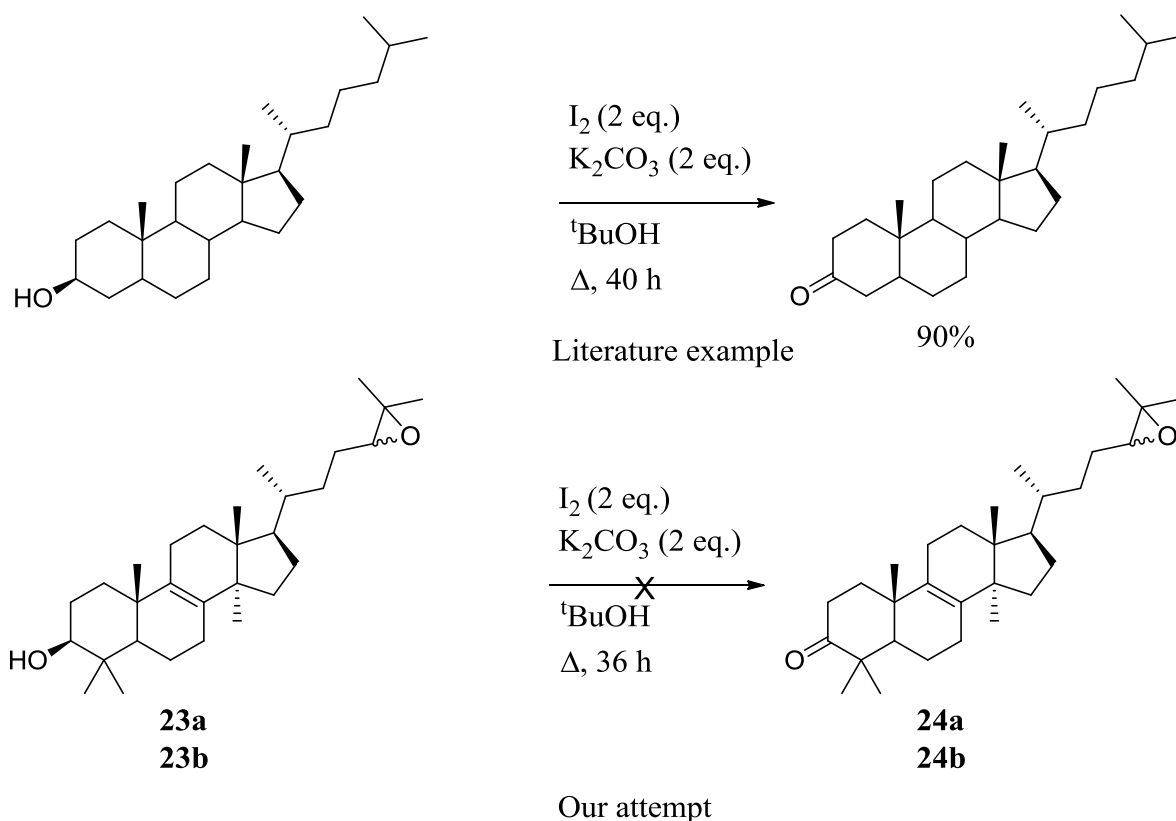


Scheme 2.12

The formation of the aldehyde **25** was evident from a new triplet at 9.77 ppm in the ^1H NMR spectrum corresponding to 24-CH, and a new multiplet between 2.25 ppm and 2.55 ppm corresponding to 23-CH₂. The absence of the 24-CH epoxide signal at 2.69 ppm in the starting epoxide **23** was also observed upon analysis of the ^1H NMR spectrum. By comparison of the ^{13}C NMR spectra, the change is also evident. A new signal at 203.3 ppm in the ^{13}C NMR spectrum of the product is clear evidence for the formation of the desired aldehyde **25**. This reaction proceeded in 73% yield and gave the alcohol analogue of the C-24 aldehyde.

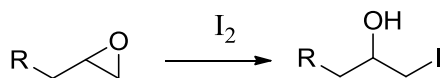
Secondly, epoxide **24**, with ketone functionality at C-3, was more challenging to synthesise. It involved the selective epoxidation of the C-24,25 alkene, as well as the selective oxidation of the sterically hindered C-3 alcohol, without allylic oxidation or opening of the epoxide ring. Therefore, a synthetic strategy was devised by a trial and error approach to this sequence of synthetic steps.

Our initial aim was to synthesise the epoxide prior to the secondary alcohol oxidation. A literature search²³ then revealed a procedure for the selective oxidation of the secondary alcohol in steroids using a mild procedure involving iodine and potassium carbonate.



Scheme 2.13

This reaction was carried out over a 36 hour reflux period. After this time ^1H NMR analysis showed the reaction had only gone $\sim 10\%$ to completion. Our judgement of this reaction was that the epoxide was in some way interfering with the alcohol oxidation, given that the literature example²³ was an unfunctionalised steroid. It is known that epoxides can be converted into halohydrins by means of elemental halogen (Scheme 2.14).²⁴ This is a sluggish reaction, often requiring a catalyst. However, under our reaction conditions of refluxing over a 36 hour period, it is possible that this transformation had taken place.

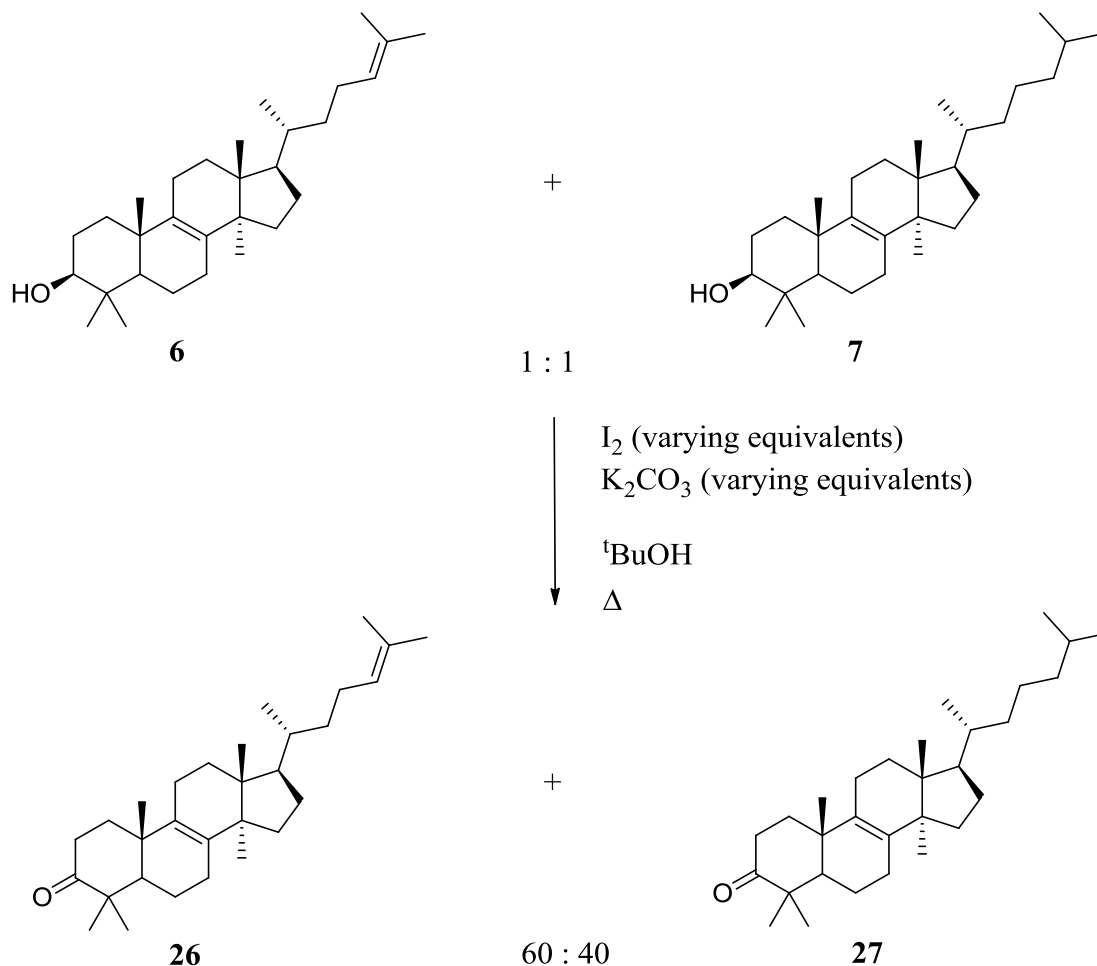


Scheme 2.14

We decided to proceed with a different synthetic strategy. This involved oxidising the commercial lanosterol **6** and dihydrolanosterol **7** mixture prior to epoxidation. Literature precedent indicated two equivalents of iodine and potassium carbonate were sufficient for the oxidation of the secondary alcohol. In our first attempt of this reaction, two equivalents of both reagents were employed over a 36 h time period, resulting in 35% yield of product. The reaction time was then increased to 48 h, which increased the yield to 57%. However, our

optimisation work involved shortening the reaction time from 48 to 36 hours using four equivalents of the oxidant with approximately the same yield of product (Table 2.4).

Table 2.4

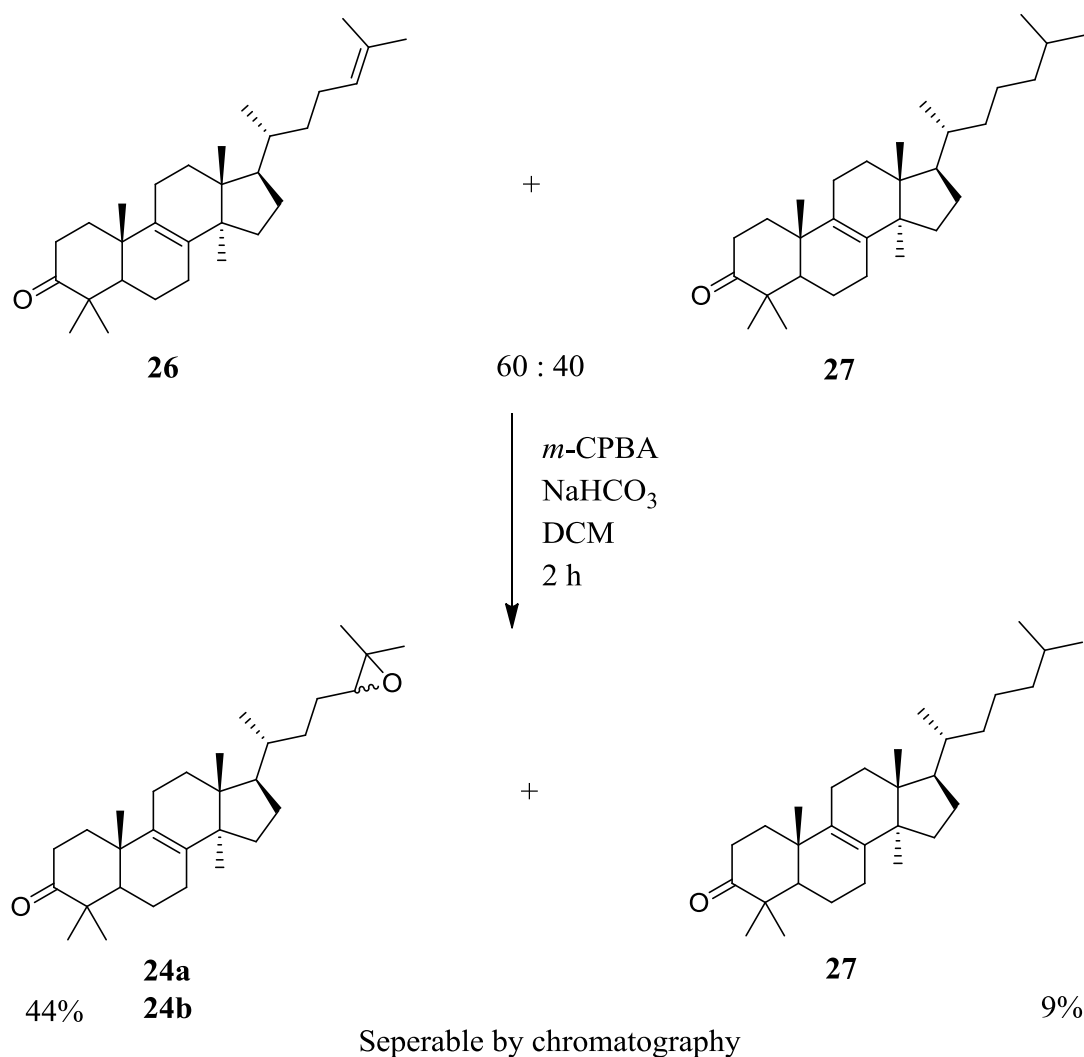


Entry	I_2 equivalents	K_2CO_3 equivalents	Time	Yield
1	2	2	36 h	35%
2	2	2	48 h	57%
3	4	4	36 h	50%

Although this diminished the reaction yield by 7%, the shortening of the reaction time from 48 hours to 36 hours enabled the reflux to be carried out through one night instead of two. This was considered significantly advantageous, from both a laboratory safety and time management point of view.

The oxidation is indicated by the loss of the 3-CH signal at 3.24 ppm in the ^1H NMR spectrum of the starting alcohol mixture **6** and **7**.

While several attempts to separate the two ketones **26** and **27** proved unsuccessful, the mixture could be separated when the side chain of **26** had been epoxidised at the C-24,25 alkene (Scheme 2.15).

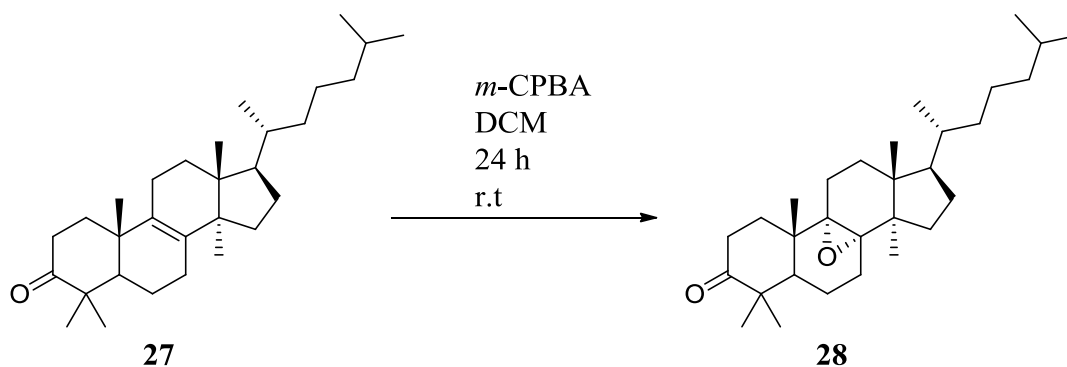


Scheme 2.15

This was the final successful route which led to the synthesis of the keto epoxide **24** in 44% yield after purification. The ^1H NMR spectrum of the product showed the triplet at 2.69 ppm corresponding to the epoxide 24-CH as its only distinguishable peak. The signal at 217.9 ppm in the ^{13}C NMR spectrum was evidence that the ketone functional group at C-3 was still intact. The ^{13}C NMR spectrum gave further support to the structure of the product with the epoxide carbons appearing at ~58 ppm and ~64 ppm.

This was a big milestone in the project as it involved the combination of our most biologically significant results to date. The epoxide in the side chain was retained in this new product and the ketone functionality had been introduced.

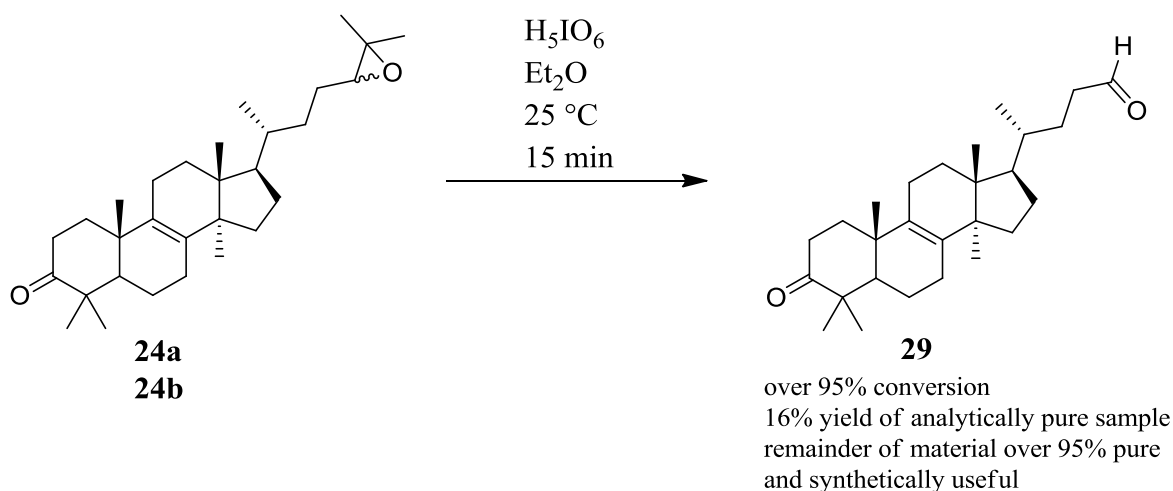
The side product of this reaction, ketone **27**, was characterised by ^1H and ^{13}C NMR analysis, the main characteristic signal being a peak at 218.0 ppm which corresponds to the ketone at C-3. This product was carried on to a further step and reacted with *m*-CPBA to epoxidise the C-8,9 double bond, in order to synthesise **28** (Scheme 2.16). However, purification was found to be very difficult, requiring repeated column chromatography. The ^1H NMR analysis described was carried out on a sample containing minor residual amounts of *m*-CPBA. Further purification and analysis was not possible due to insufficient quantities of material.



Scheme 2.16

When the keto epoxide **24** was synthesised and available for biological testing, the next step was to synthesise the aldehyde at the C-24 position **29** from this substrate. This would lead to the completion of the library of C-24 aldehydes with acetate **21**, alcohol **25** and ketone **29** groups at C-3 respectively.

The established oxidative cleavage method was used for the synthesis of aldehyde **29** (Scheme 2.17).



Scheme 2.17

Again, the oxidative cleavage reaction took place over a short time period of 15 min. The desired aldehyde was formed in 16% yield after repeated chromatography. The formation of the aldehyde **29** was evident from a new triplet at 9.78 ppm in the ^1H NMR spectrum corresponding to 24-CH. In this case, the 23-CH₂ multiplet in the 2.20 ppm – 2.60 ppm region was obscured by the overlapping 2-CH₂ multiplet, which appears with greater downfield shift due to its proximity to the ketone at C-3. The disappearance of the 24-CH epoxide signal at 2.69 ppm in the starting material **24** was also observed upon analysis of the ^1H NMR spectrum. By comparison of the ^{13}C NMR spectra, the change is also apparent. A new signal at 203.3 ppm in the ^{13}C NMR spectrum of the product is clear evidence for the formation of the desired aldehyde **29**.

While the purified yield of the C-3 alcohol analogue **25** of these C-24 aldehydes is quite good (73%), the isolated yields of the acetate **21** and ketone **29** analogues were rather disappointing, at 12% and 16% respectively. Encouragingly, this is not due to failed conversion of epoxide to aldehyde, as $\geq 95\%$ conversion is observed by analysis of the ^1H NMR spectra, but instead is a purification issue. As the R_f values of the side products are very close to that of the desired product on a TLC plate, the isolated yield of analytically pure sample of aldehyde is, in this case, 12 – 16%. The other ~80% of sample has a purity of $\geq 95\%$, which was used in the synthetic work described in this thesis.

The formation of side products in this oxidative cleavage reaction could be playing a role in diminishing the yield. By analysis of the ^1H NMR spectrum of the crude material, only the desired aldehyde is observed. However, trace amounts of side products are seen as faint spots on a TLC plate. One such side product is ketone **30** (Figure 2.22), which was formed in 1% yield and was isolated from the column as a fraction preceding the desired aldehyde.

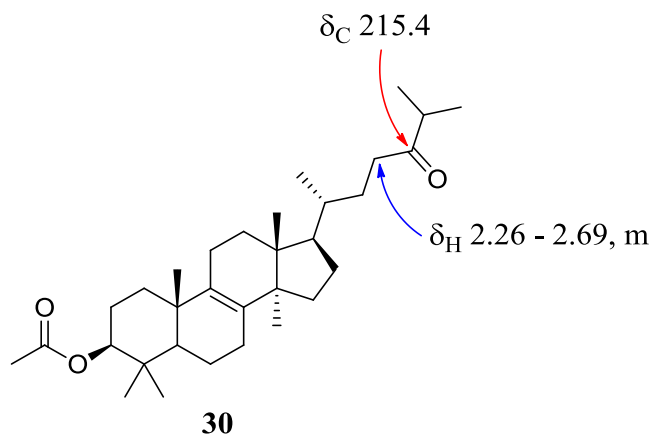


Figure 2.22

This is a side product from the oxidative cleavage reaction of **22**. The ^1H NMR spectrum of this isolated side product showed the characteristic multiplet between 2.26 ppm and 2.69 ppm which occurs when 23- CH_2 is adjacent to a carbonyl functionality. However there was no trace of an aldehyde signal at ~ 9.7 ppm in the ^1H NMR spectrum. This led us to believe a ketone rather than an aldehyde was present at C-24, and ^{13}C NMR analysis confirmed this. The signal at 215.4 ppm in the ^{13}C NMR spectrum showed a ketone functionality was present. The side chain structure was then confirmed by revisiting the ^1H NMR spectrum and noting the recurrence of the 26- CH_3 or 27- CH_3 as a distinct doublet at 1.09 ppm, indicating that the C-24,25 bond had not been cleaved in this case, and leading us to the tentative assignment²⁵ of the structure of this side product as **30** (Figure 2.22).

Mechanistically, we propose that this product is formed in a similar fashion to the aldehyde. The acid ring opening of the epoxide, followed by insertion of the iodine complex to form the five membered ring system remains the same. However, the concerted collapse proceeds *via* a different pathway leading to the formation of the ketone and not cleavage of the C-C bond to form the aldehyde (Figure 2.23).

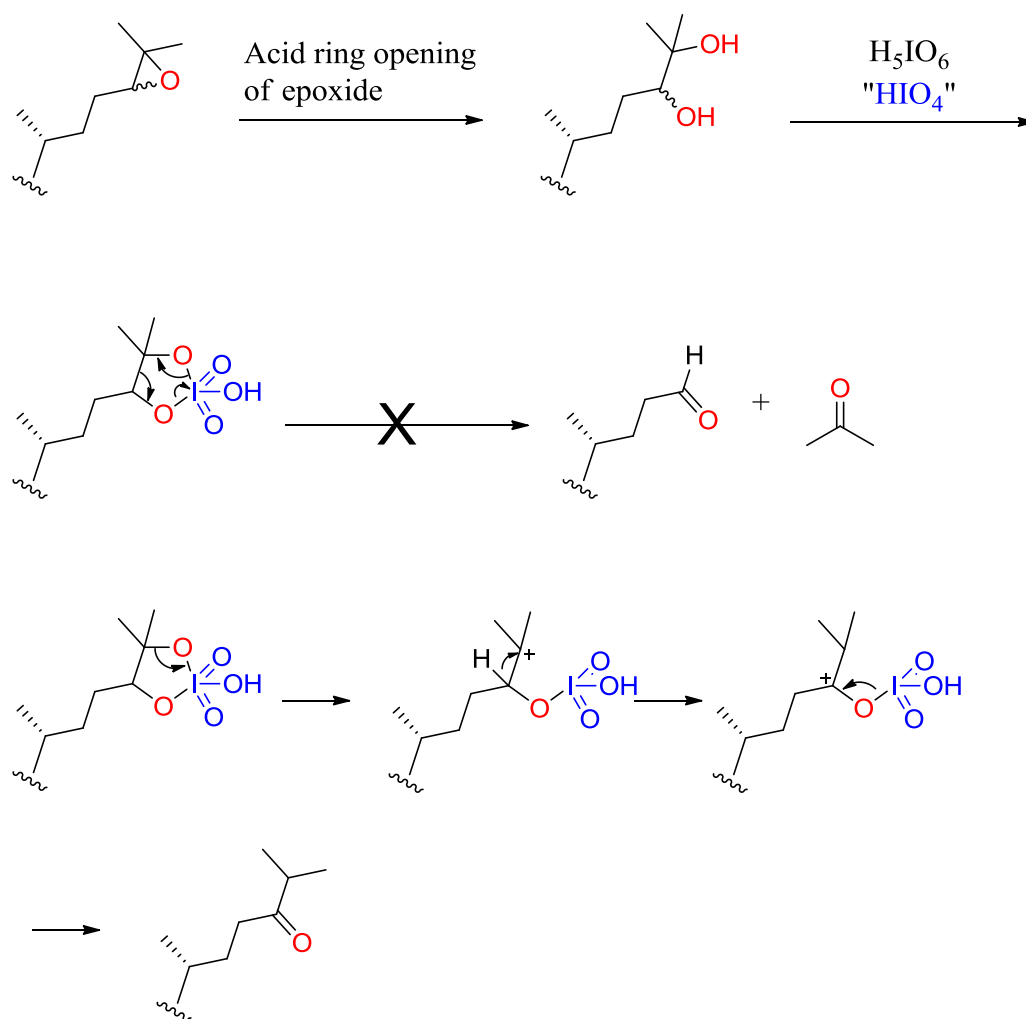


Figure 2.23

2.3.7 Synthesis of 3 β -acetoxy-lanost-8,9-epoxide **1**, and 3 β -acetoxy-5 α -lanost-7,9(11)-diene **2**

As discussed in the “Target Transformations” Section 2.32, the conjugated diene of the central ring structure was a desirable moiety to synthesise, as it is present in four isolates from *A. camphorata*. Compound **2** (Figure 2.24) had been previously synthesised in the group and contained the diene motif across the B and C rings. Unfortunately, this compound had shown poor activity against the *Sf9* insect cells. Therefore, our aim was to synthesise this compound again, in order to test it against the CaCo-2 cells and the U937 cells, with a view to expanding a series of conjugated dienes if **2** showed good activity.

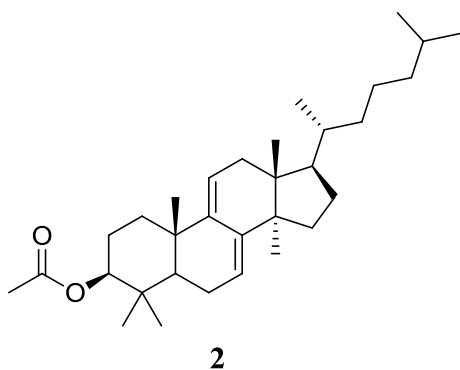
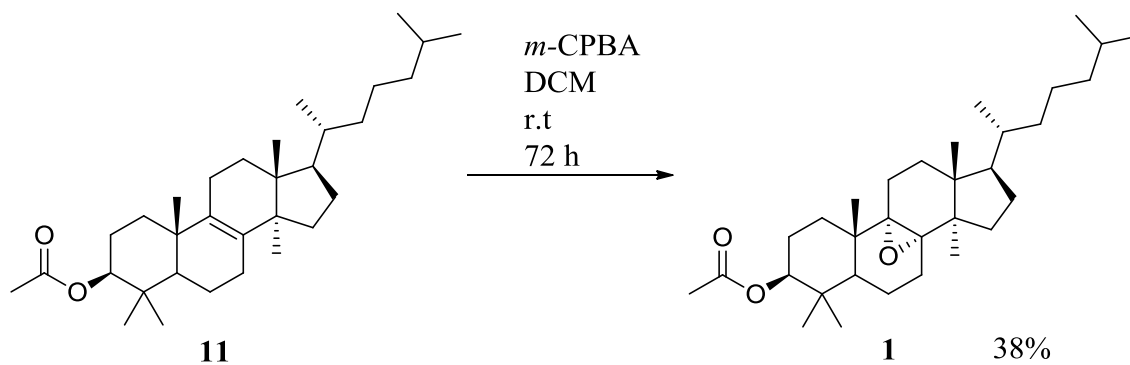


Figure 2.24

Epoxidation at C-8,9 is the first step in the strategic transformation of dihydrolanosterol acetate **11**, via epoxide **1**, to diene **2** (Scheme 2.18 and Scheme 2.19).

Dihydrolanosterol acetate **11** was reacted with *m*-CPBA and epoxidation occurred at the only available position, the alkene at C-8,9. Purification by repeated column chromatography yielded the pure product **1** as a white solid (38%). The ^1H NMR spectrum of **1** was indistinguishable from starting material **11** due to quaternary carbons at the site of reactivity in both the starting material and product. The ^{13}C NMR spectrum gave good evidence for the transformation, showing an upfield shift of the C-8 and C-9 signals from 134.2 ppm and 134.5 ppm to 68.0 ppm and 70.5 ppm respectively.



Scheme 2.18

This reaction took 72 hours to go to completion and required 4.2 equivalents of *m*-CPBA. This result is not unexpected, as the C-18, C-19 and C-30 methyl groups are causing steric hindrance near the site of reactivity. It is the orientation of these methyl groups which determines the stereoselectivity of the reaction. The methyl groups at C-18 and C-19 are both

lying on the β face, thus inhibiting free approach of the peroxy acid reagent. Though 30-CH₃ and 5-CH are both lying on the α face, the steric availability of this side of the steroid is far greater as a CH group exerts less steric hindrance than a CH₃ group. Therefore, α face attack predominates and the α epoxide **1** is formed exclusively.

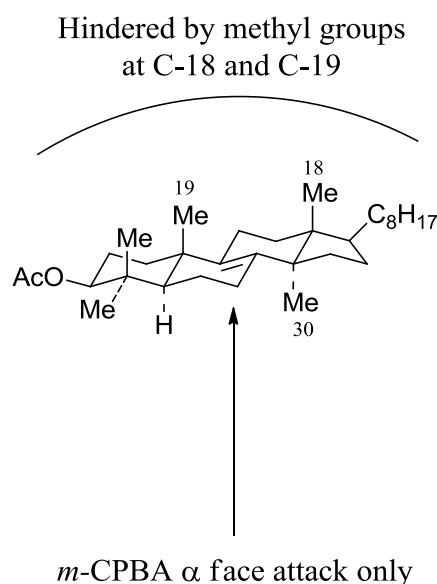
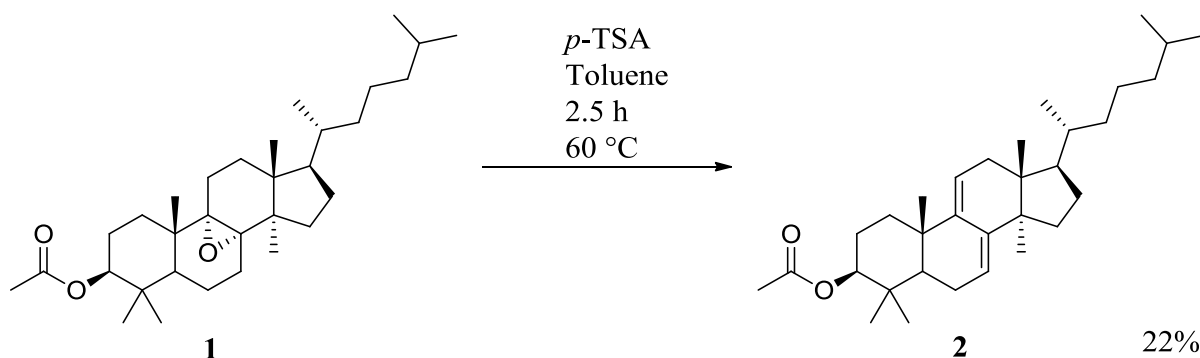
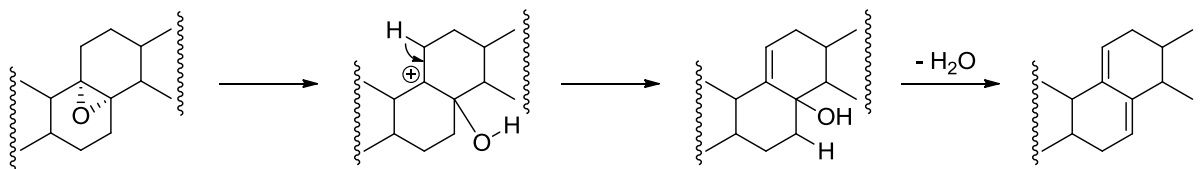


Figure 2.25

The ring opening of the C-8,9 epoxide **1** is the next step in the synthesis of the conjugated diene. *p*-Toluenesulfonic acid was used to ring open the epoxide, which was followed by formation of diene **2** via an allylic carbocation (Scheme 2.19 and Scheme 2.20).^{26,27}



Scheme 2.19



Scheme 2.20

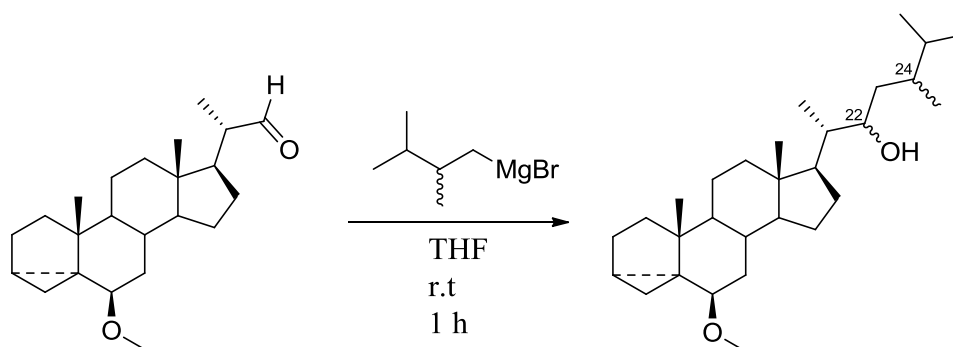
Formation of the desired product is evident from analysis of the ^1H NMR spectrum. The acetyl group at C-3 is still present due to the 1H doublet of doublets at 4.52 ppm. New signals at 5.34 ppm and 5.46 ppm correspond to the vinylic protons 11-CH and 7-CH respectively. The ^{13}C NMR spectrum showed C-11 and C-7 shifting downfield from 21.5 ppm and 23.3 ppm to 116.6 ppm and 119.8 ppm. These characteristic signals of vinylic protons are good evidence for the formation of the conjugated diene moiety.

The reaction proceeded with $\sim 60\%$ conversion to the desired product. This is evident from the characteristic shift of the 3-CH signal in the ^1H NMR spectra. This signal is shifted slightly downfield from 4.46 ppm in the starting material, to 4.52 ppm in the product. Purification required repeated chromatography, yielding the product **2** in 22% yield. This reaction would need to be optimised in future work, to increase the percentage conversion of starting material to product. Optimisation work could include use of additional equivalents of *p*-toluenesulfonic acid, or longer reaction times.

This compound was characterised and prepared for a future screening. It also had the potential to be further derivatised, as the conjugated diene introduces new reactive sites on both rings. However, the initial objective would be to test the parent compound before any derivitisation would take place. Unfortunately, when the compound was sent for testing, it was found to show unusual poor solubility in the appropriate solvents, for example, ethanol, or dimethylsulfoxide. Therefore, no biological results were obtained for this compound.

2.3.8 Grignard reactions on aldehyde **21**

As previously discussed, the C-24 aldehyde moiety in the lanostane structure was highly synthetically useful in terms of Grignard reactions, Wittig reactions, and reductive aminations (Figure 2.15). Takatsuto *et al.* report a Grignard reaction on a protected cholestane steroid to yield a variety of products (Scheme 2.21).²⁸



Isomers were formed in the following yields:

(22*S*,24*S*) isomer, 19%; (22*S*,24*R*) isomer, 46%; inseparable mixture of (22*R*,24*R*) and (22*R*,24*S*) isomers, 7%.

Scheme 2.21

The Grignard reaction could also be applied to our C-24 aldehydes in the lanostane derivatives. As our side chain functionalisation had thus far incorporated oxygen groups at carbons 24 and 25, a carboxylic acid or ester at C-26 was our next objective, as certain isolates from *A. camphorata* bear an ester moiety at this position in the side chain (Figure 2.26). Selective activation of one of the 26-CH₃ or 27-CH₃ groups to selectively form one ester seemed, at first, to be an unrealistic goal. However, aldehyde **21** opened up this area of chemistry for us. By use of a Grignard reaction²⁹ we could choose a suitable precursor with an appropriate side chain to attach at this position.

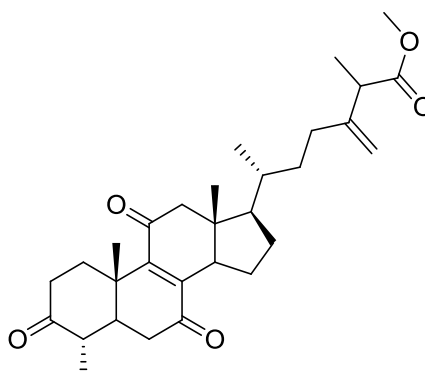
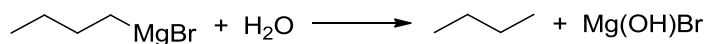


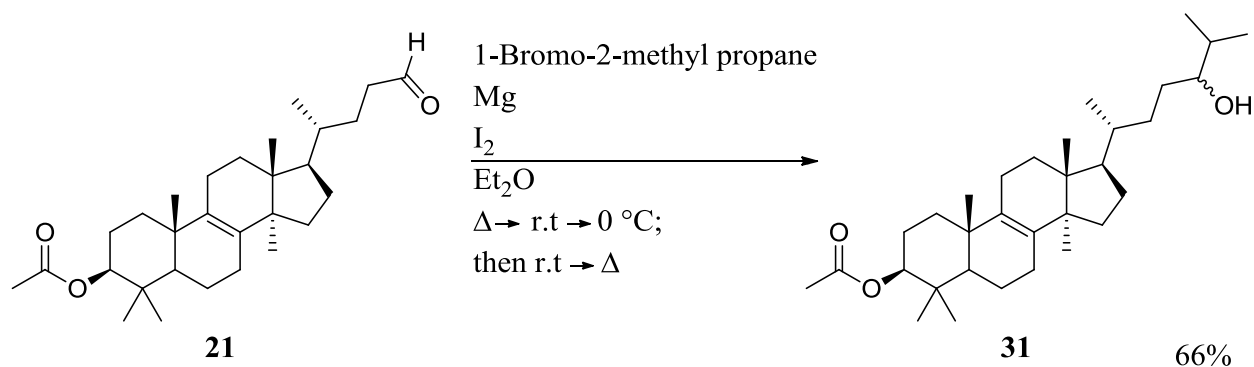
Figure 2.26

The first Grignard reaction that was attempted was a test reaction, in order to establish the optimum conditions under which this transformation could be achieved. Aldehyde **21** was reacted with 1-bromo-2-methyl propane to form 3 β -acetoxy-5 α -lanost-8-ene-24-ol **31**. All glassware to be used for the Grignard reaction was dried overnight at 150 °C prior to use, as traces of water could impede the reaction by reacting with the Grignard reagent to form alkanes, an example of which is shown in Scheme 2.22.



Scheme 2.22

The standard method of using iodine to initiate the reaction was used. The iodine helps to remove an outer layer of oxide from the magnesium, thus exposing fresh magnesium surface and improving the rate of formation of the Grignard reagent, and consequently improving the rate of reaction. The Grignard reagent was seen to form spontaneously when the bromide was added to magnesium and iodine in diethyl ether, as reflux initiated of its own accord.

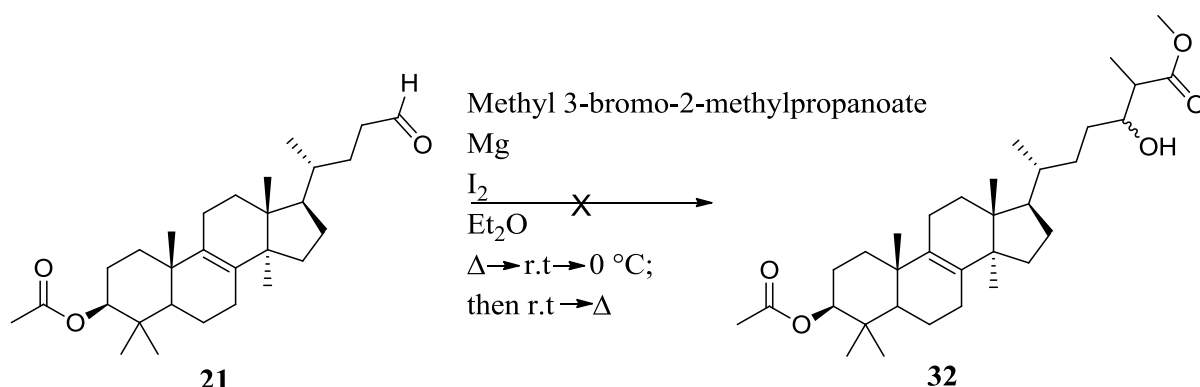


Scheme 2.23

The desired product **31** was formed as a dark orange oil in 66% yield (Scheme 2.23). Confirmation of its formation is seen in the ¹H NMR spectrum. A new signal is observed as a multiplet between 3.52 ppm and 3.72 ppm. This multiplet is assigned as the hydrogen of the new alcohol group at C-24. The doublet of doublets at 4.50 ppm is consistent with the presence of the acetyl group at C-3. The ¹³C NMR spectrum shows new signals at 71.4 ppm

and 71.7 ppm respectively, which correspond to 24-CH *R* or *S* isomers. 3-CH appears at 80.2 ppm, showing the presence of the acetyl group.

Given the success of this trial reaction, we focussed on our main target, which was to introduce an ester containing group into the side chain, similar to those seen in the most active compounds from *A. camphorata*. A Grignard reaction was performed using a different bromide to achieve this transformation. Aldehyde **21** was reacted with methyl 3-bromo-2-methylpropanoate in an attempt to synthesise **32** (Scheme 2.24).



Scheme 2.24

Unfortunately this reaction was unsuccessful following several attempts. In this case, there were no signs of initiation of the reaction to form the Grignard reagent as reflux did not occur spontaneously, as was seen in the previous reaction to synthesise **31**. A heat gun was employed to allow reflux to occur, and the solution was maintained at reflux using an oil bath. However, despite our best efforts, it was not apparent that the Grignard reagent had formed by observation of the orange colour of the reaction solution. As this was the first attempt at this reaction, we were unsure of the exact colour change, but after an appreciable amount of time to allow Grignard reagent formation the reaction was proceeded with, to establish if any products could be isolated from the reaction.

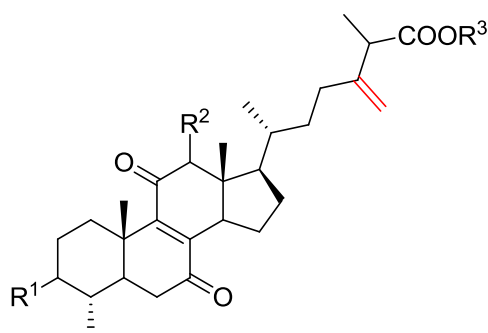
Of course, the ¹H NMR spectrum revealed a mixture of starting aldehyde **21** and methyl 3-bromo-2-methylpropanoate with no evidence of product formation. The starting aldehyde is still present due to the characteristic triplet at 9.77 ppm in the ¹H NMR spectrum. An unidentified alkene signal at 5.12 ppm is also observed in the spectrum. An excess of bromide

starting material in relation to the steroid component is observed. Characteristic signals for methyl 3-bromo-2-methylpropanoate in the ^1H NMR spectrum are a 3H doublet at 1.23 ppm, a 1H quartet at 2.91 ppm, a 2H doublet of doublet doublets at 3.53 ppm, and a 3H singlet at 3.73 ppm.

Unfortunately, after several attempts, this reaction remained unsuccessful. It is interesting that the trial reaction with 1-bromo-2-methyl propane yielded the desired product (Scheme 2.23), while methyl 3-bromo-2-methylpropanoate did not show any signs of forming the Grignard reagent. This may be due to increased steric bulk of the latter reagent, or an electronic effect imposed by the ester group. Nonetheless, this reaction will need to be repeated in future work, possibly with alternative bromides, to introduce an ester containing group into the side chain.

2.3.9 Wittig reaction on 3 β -acetoxy-5 α -lanost-8-ene-7,11,24-one-25-ol 14

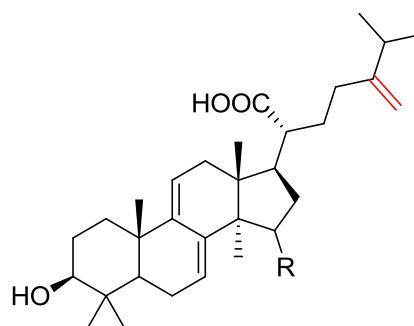
As can be seen above, the Grignard route for introduction of an alternative side chain was unsuccessful for the ester derivative. Therefore, our attention turned to the incorporation of a side chain by means of a Wittig reaction. As seven of the eight isolates from *A. camphorata* bear an alkene moiety at C-24 (Figure 2.27), it was a target for us to try to incorporate this functional group *via* Wittig chemistry.



Isolate 1: R¹ = (=O), R² = H, R³ = CH₃

Isolate 5: R¹ = (=O), R² = R³ = H

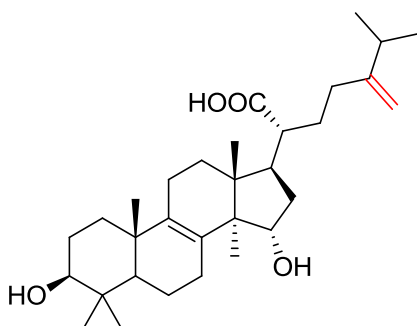
Isolate 7: R¹ = R² = β -OH, R³ = H



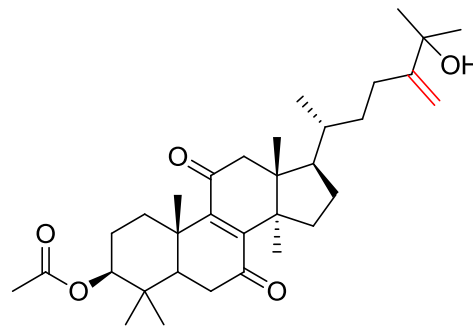
Isolate 2: R = H

Isolate 3: R = α -OAc

Isolate 6: R = α -OH



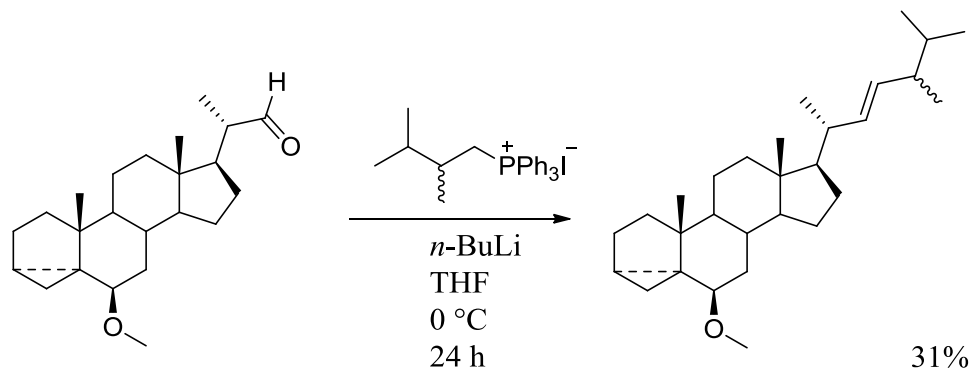
Isolate 8



Target Compound

Figure 2.27

Successful Wittig reactions have been carried out by O'Connell *et al.*⁷ on a protected cholestane steroid containing an aldehyde at C-22, as shown in Scheme 2.25.



Scheme 2.25

Upon consideration of this reaction, we determined that a C-24 aldehyde would be a less advantageous precursor, due to the fact that the terminal isopropyl group could not be achieved, and a more useful starting material would contain a ketone in place at C-24, adjacent to the desired isopropyl group (Figure 2.28).

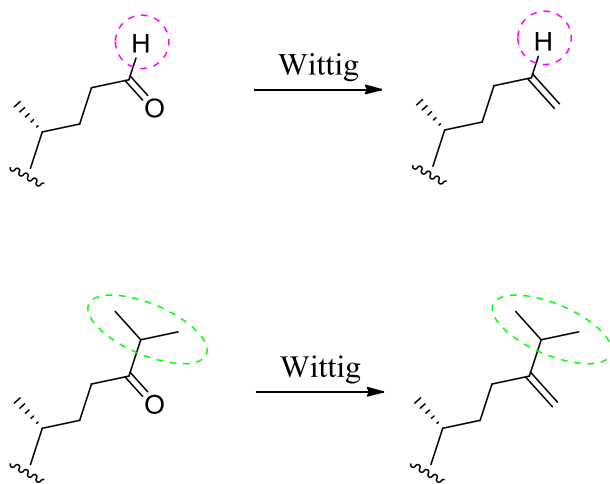
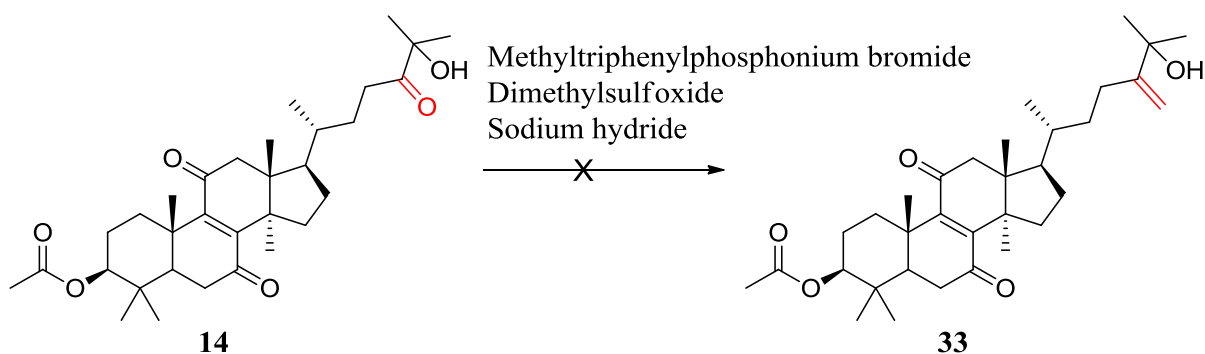


Figure 2.28

We had previously synthesised a compound containing a ketone at C-24, **14**. Therefore, we turned our focus to a Wittig reaction to possibly introduce an alkene at this position. It was envisaged that the steric availability of this ketone at C-24 would allow it to react in preference to the other two ketones at C-7 and C-11.

Triketone **14** was used as substrate in a Wittig reaction (Scheme 2.26). The reaction was carried out using sodium hydride, dimethyl sulfoxide and methyltriphenylphosphonium bromide.^{30,31} Analysis of the ¹H NMR spectrum of the crude product showed no evidence of the formation of the desired product **33**, and also no evidence of starting material **14**. The crude product of the reaction was determined to be a complex mixture of unidentifiable products. It is possible that the site of reactivity at the C-24 ketone is too sterically hindered for the bulky triphenyl phosphonium reagent to approach. A longer reaction time, or increased equivalents of the Wittig reagent may lead to a successful outcome in future work, however, in our case the reaction was not pursued further due to insufficient starting material, and other areas of the project requiring our attention.



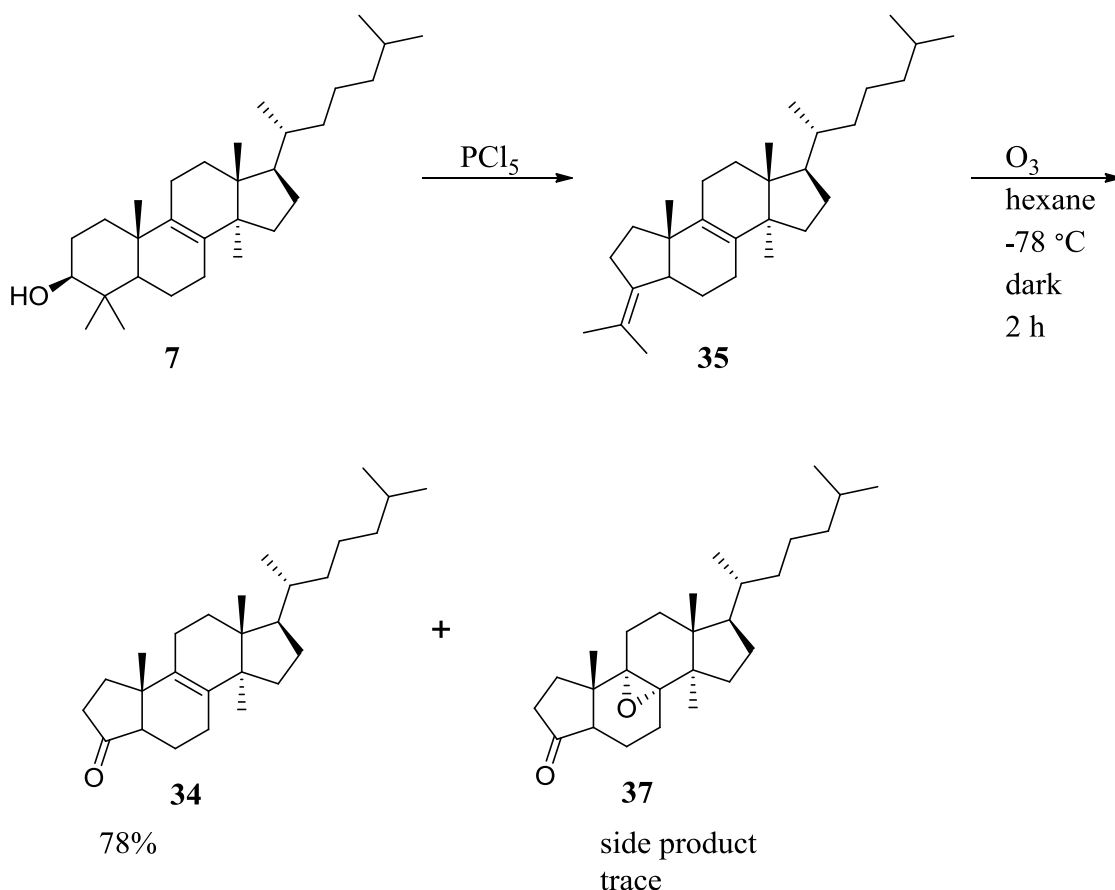
Scheme 2.26

2.3.10 A ring contraction of dihydrolanosterol 7

Following on from our side chain functionalisation and modification of the B and C rings, our interest turned to variations on the A ring of the lanostane structure. Although modifications had already been made at C-3, including the introduction of ester and ketone functionalities, we saw the opportunity to contract the A ring to a 5 membered ring,^{32,33} with scope for further

derivitisation. This body of work would add diversity to our collection of lanosterol oxidation products and possibly provide interesting compounds for biological evaluation.

Paryzek *et al.* studied the solvent effect in reactions of tetrasubstituted triterpenoidal alkenes with ozone, and reported an A ring contraction procedure using phosphorous pentachloride.³² The A ring contracted product was subsequently treated with ozone as shown in Scheme 2.27.

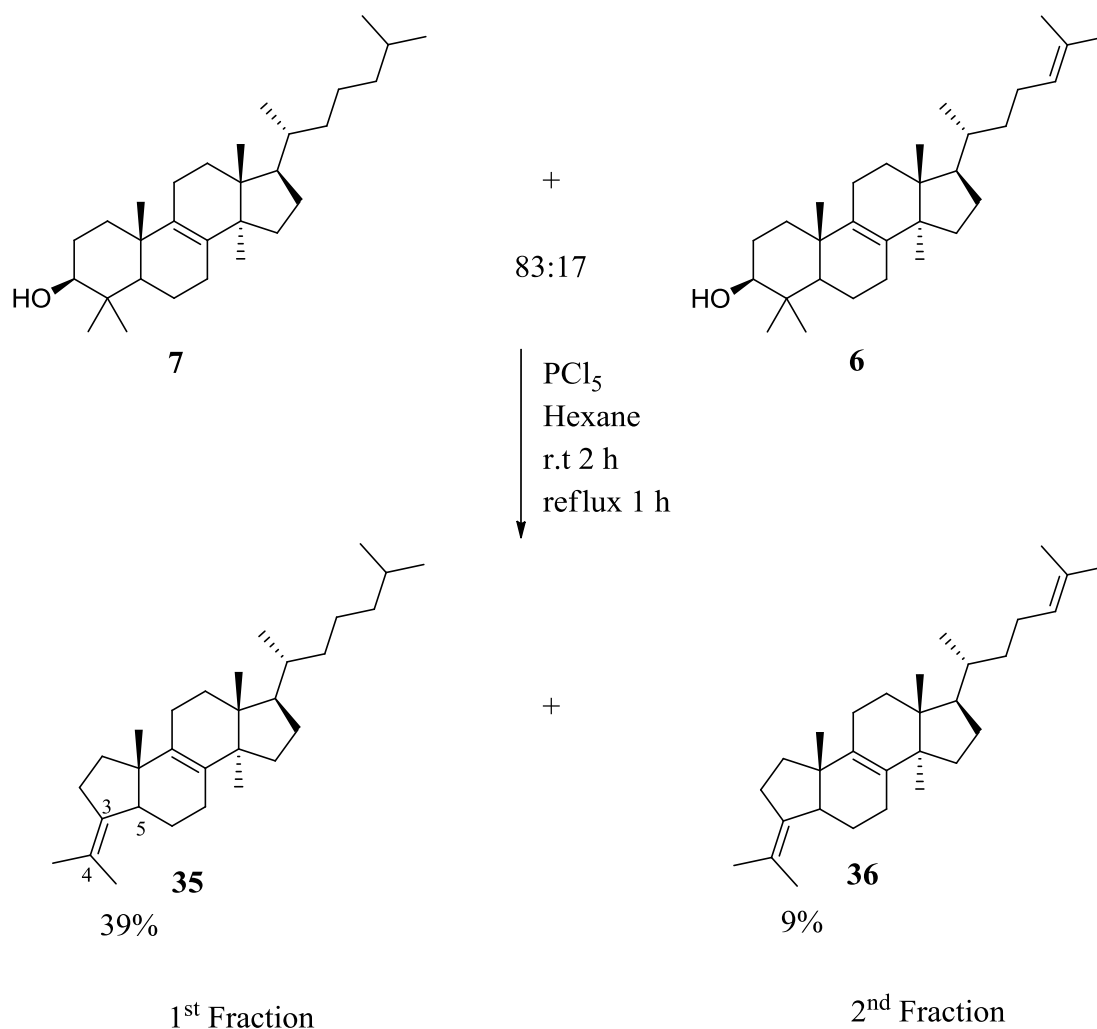


Paryzek does not report exact conditions for the A ring contraction in his publication. Details of this reaction can be found in a communication by Huffman.³¹

Scheme 2.27

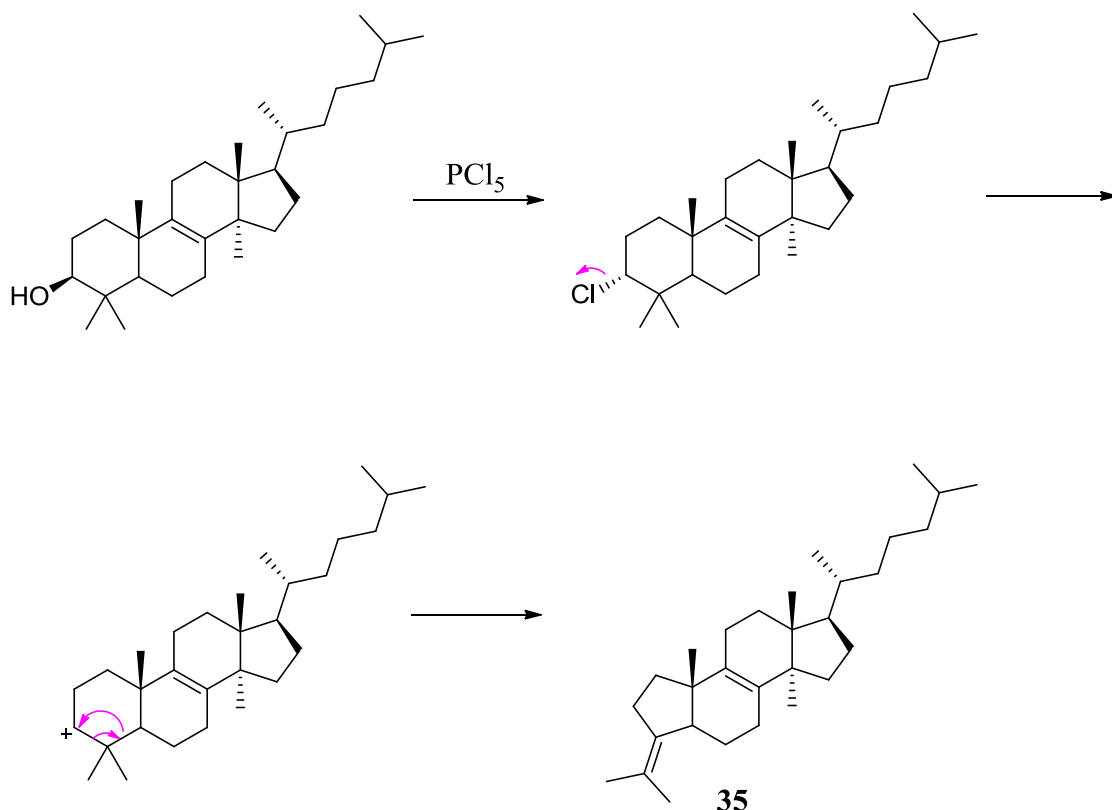
The ketone product of this ozonolysis reaction **34** was an initial target for our series of A ring contracted compounds, with a view to further functionalising the C-8,9 alkene and synthesising **37** (Scheme 2.27) which is an extremely useful analogue for our research, as will be discussed in due course. The initial step in the synthesis of ketone **34** was the A ring contraction.

Thus, an 83:17 mixture of dihydrolanosterol **7** and lanosterol **6** was reacted with phosphorous pentachloride in a retropinacolic rearrangement reaction (Scheme 2.28) to yield 3-isopropyliden-14 α -methyl-A-nor-5 α -cholest-8-ene **35** and 3-isopropyliden-14 α -methyl-A-nor-5 α -cholest-8,24-diene **36**.



Scheme 2.28

Purification by column chromatography afforded **35** in 39% yield as the first fraction from the column. This interesting reaction proceeds *via* the mechanism proposed below in Scheme 2.29, beginning with chlorine displacement of the alcohol group, followed by rearrangement of the A ring.



Scheme 2.29

Verification of product formation was seen in the ^1H and ^{13}C NMR spectra. There were few characteristic signals for the product in the ^1H NMR spectrum, as there are no heteroatoms present in its structure. However, the absence of the doublet of doublets at 3.23 ppm corresponding to 3-CH of the starting material is good evidence for the formation of the product. The ^{13}C NMR spectrum is much more informative. A second alkene in the structure is evident with new signals at 121.6 ppm and 135.2 ppm, corresponding to the new C-4 and C-3 respectively. The C-8,9 alkene is also retained, with signals at 133.6 ppm and 134.2 ppm.

The second fraction from the column was 3-isopropyliden-14 α -methyl-A-nor-5 α -cholest-8,24-diene **36**, formed in 9% yield. The additional signals at 125.3 ppm and 130.9 ppm in the ^{13}C NMR spectrum, correspond to C-24 and C-25 respectively.

When the A ring contraction was complete, the possibility for exploitation of the diene of **35** led to the emergence of a target molecule containing ketone and epoxide functionalities (Figure 2.29). This target molecule could be synthesised through an ozonolysis reaction to form **34** followed by epoxidation of the C-8,9 alkene to yield **37**.

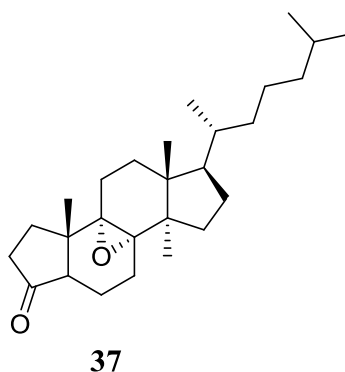
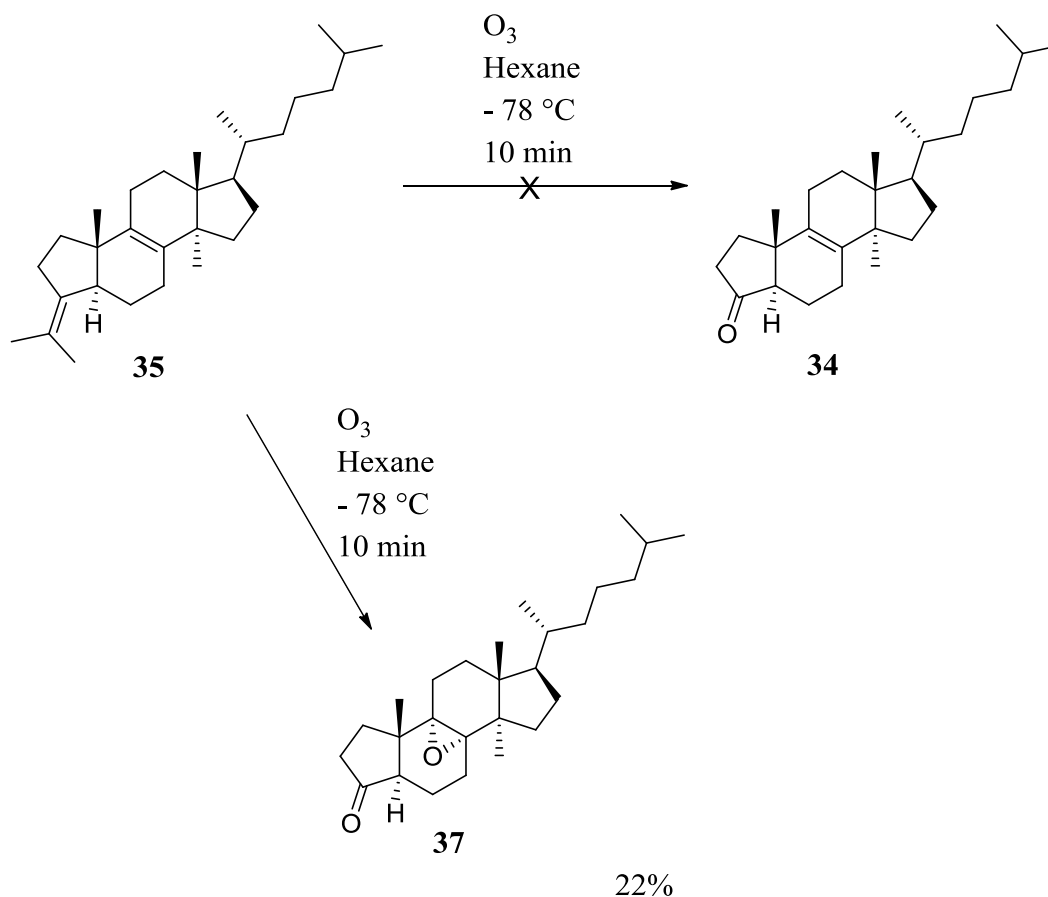


Figure 2.29

The first step in the synthesis of **37** involved the cleavage of the A ring alkene bond *via* ozonolysis.³² Oxygen was passed through the reaction set up before ozone as a precautionary measure to identify any leaks in tubing. Once the set up was satisfactory the ozone generator was switched on and ozone was allowed to pass through the reaction vessel. 10% potassium iodide solutions in water were used to destroy excess ozone emerging from the reaction set up. Upon reaction completion, triphenylphosphine was added to the reaction mixture, in order to remove any residual ozonide. The crude ozonolysis product was purified by column chromatography. Inspection of the ^1H and ^{13}C NMR spectra of the purified material revealed an unexpected result. The major product of the reaction was not **34** as anticipated, but rather **37**, which was isolated as a clear oil in 22% yield containing trace amounts of triphenylphosphine oxide (Scheme 2.30).

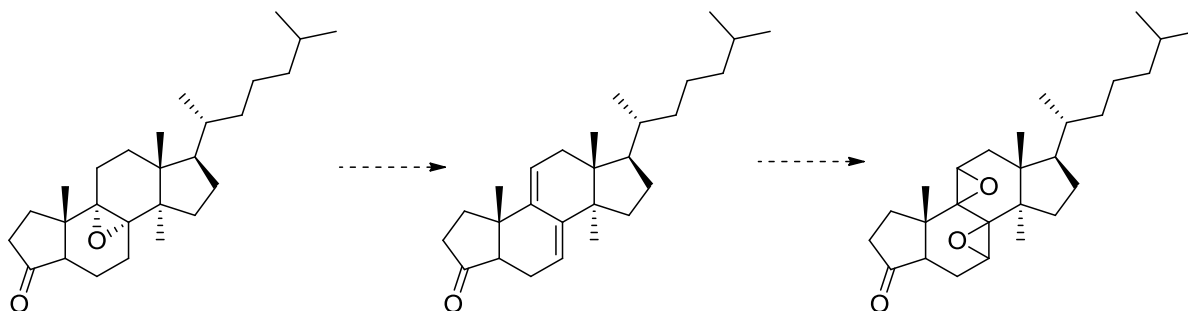


Scheme 2.30

The formation of the epoxide in this reaction was a very positive result, as our desired product **37** had been synthesised in a one pot reaction, eliminating the need for a second synthetic step.

The ^1H NMR spectrum revealed a 1H doublet of doublets at 2.68 ppm which corresponds to $5\alpha\text{-CH}$. This downfield shift is good evidence for the presence of the ketone at C-3. The ^{13}C NMR spectrum, however, was more elucidatory. It was quite evident, upon first inspection, that the C-8,9 functionality had been lost, due to complete disappearance of the alkene carbon signals at 133.6 ppm and 134.2 ppm in the spectrum. The appearance of two new quaternary carbon signals at 68.6 ppm and 69.3 ppm gave rise to the proposal that the epoxide had been formed across this alkene bond, and high resolution mass spectrometry confirmed this theory. The signal at 217.4 ppm corresponds to the ketone in the contracted A ring.

Though non polar solvents such as hexane have been shown to yield the enone as the major product of the reaction,³² in our attempt, this was not the case. The formation of **37** at this stage opened up a range of possibilities for further derivitisation and biological testing. One such possibility is the double epoxidation of the C-7,8 and C-9,11 diene as shown in Scheme 2.31, which had been undertaken on this moiety in previous work within our group.



Scheme 2.31

However, the procedure for ozonolysis requires excess triphenylphosphine to be added to the reaction mixture upon completion as it removes residual ozonide and is itself converted to triphenylphosphine oxide. This triphenylphosphine oxide is quite difficult to remove completely by chromatography, and unfortunately, in our case, limited quantities of the product did not allow the repeated chromatography necessary for purification. Therefore it was not possible to have the biological activity of **37** evaluated, due to insufficient purity caused by trace amounts of residual triphenylphosphine oxide.

2.3.11 Biological Testing 2: Lanosterol Oxidation Products

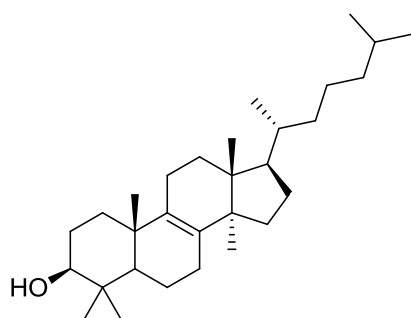
Eight more lanosterol oxidation products were evaluated in our second screen, as can be seen in Table 2.5. These results are presented in terms of IC₅₀ value and apoptosis fold increases, in addition to the percentage viability of U937 cells. The IC₅₀ measures half the maximum inhibitory concentration of a substance. The apoptosis result indicates the fold increase of visible apoptotic bodies when a particular compound is introduced to the cells in relation to the ethanol control. Any result greater than or equal to a four fold increase is considered quite significant. Therefore, a number of these results have been highlighted for their importance.

First of all, the keto-epoxide **24**, which had been specifically identified as a synthetic target, showed notable biological activity. This compound displayed a 16.4 fold increase at a concentration of 56 μ M, whereas its C-3 alcohol analogue **23** showed no apoptosis. Therefore, as we expected, the epoxide is an important feature in the side chain of the molecule, and a ketone at C-3 emphasises its biological activity.

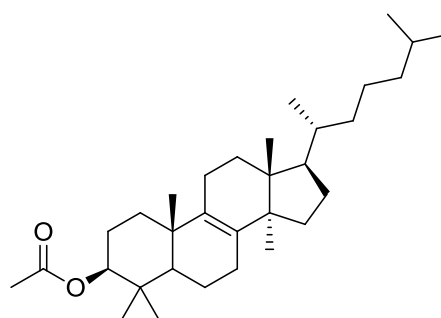
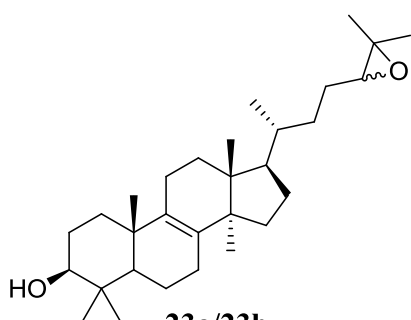
By comparison, **27** and **29**, which both also contain the ketone functionality at C-3, did not show such significant activity. A 1.2 fold increase in apoptotic bodies was seen at a concentration of 50 μ M for **27**, and a 1.8 fold increase was observed at 50 μ M of **29**. Another compound of note within this set is **30**. The ketone exhibits a 9 fold increase in apoptotic bodies at a concentration of 103 μ M. This is also quite a significant result, though not as outstanding as the 16.4 fold increase at 56 μ M displayed by **24**.

It is also worth noting the compounds which did not show particularly good biological activity. **7** and **11** displayed poor IC₅₀ values, and little or no apoptotic activity. This result is consistent with our expectations, given the low level of oxygenation in the molecules.

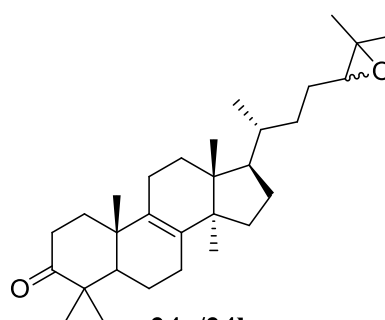
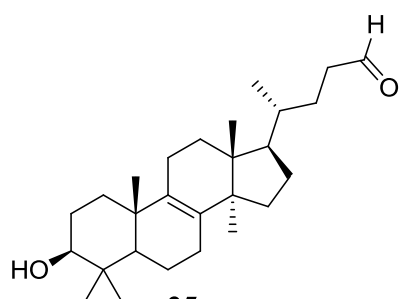
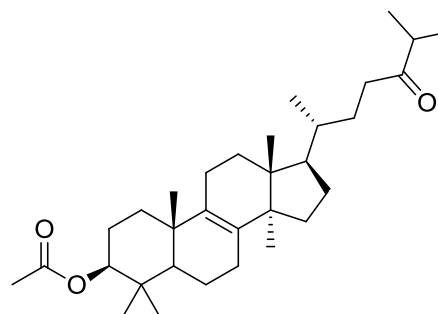
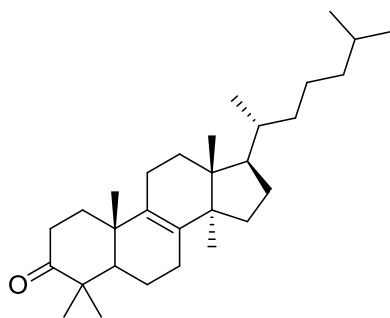
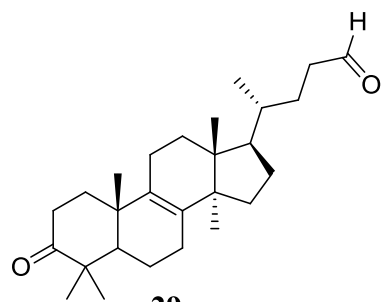
Table 2.5

**7**

Apoptosis = No

**11**Apoptosis = 425 μ M
4.9 fold increase**23a/23b**

Apoptosis = No

**24a/24b**Apoptosis = 56 & 113 μ M
16.4 and 10.7 fold increase**25**Apoptosis = 249 & 499 μ M
6.1 and 10 fold increase**30**Apoptosis = 103 μ M
9 fold increase**27**Apoptosis = 50 μ M and 100 μ M
1.2 and 2.5 fold increase**29**Apoptosis = 50 μ M and 100 μ M
1.8 and 3.9 fold increase

% Viability of U937 cells			
	200 $\mu\text{g/mL}$	100 $\mu\text{g/mL}$	50 $\mu\text{g/mL}$
7	33.86 ± 3.33		
11	51.77 ± 3.89		
23	31.79 ± 1.70		
24	7.97 ± 0.38		
25	13.98 ± 1.32		
30	7.34 ± 0.51		

% Viability of U937 cells	
	100 μM
27	31.23 ± 5.40
29	20.92 ± 5.02

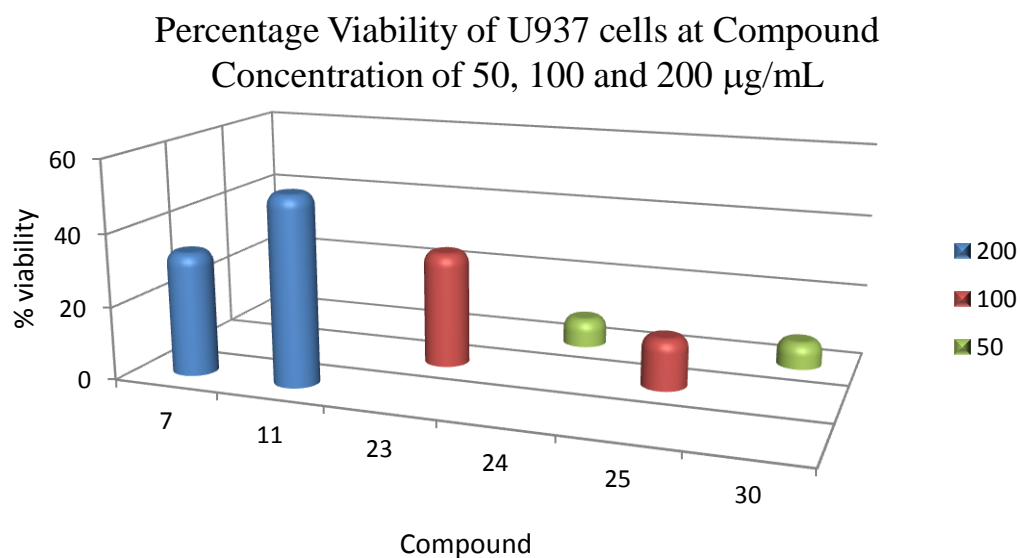
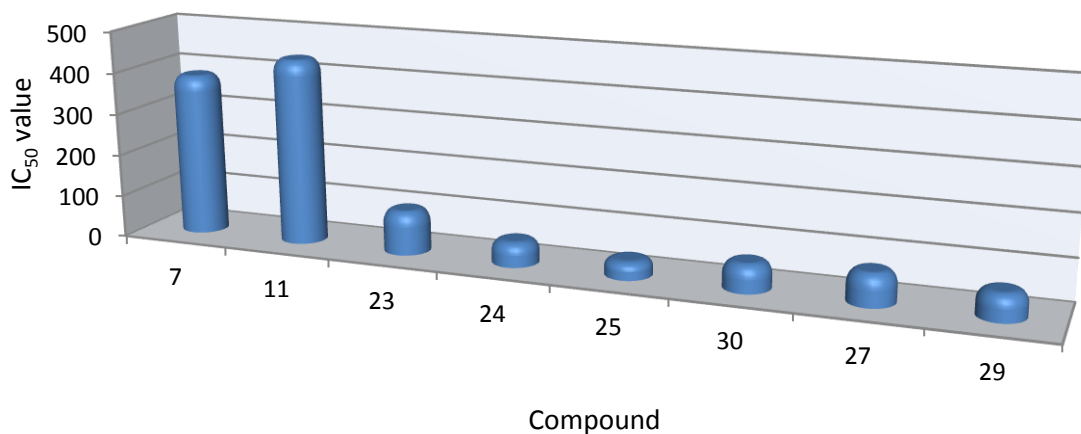


Figure 2.30: Percentage viability of U937 cells following treatment with varying concentrations of compounds **7**, **11**, **23**, **24**, **25** and **30**.

Table 2.6

	IC ₅₀ values in U937 cells	
	$\mu\text{g/mL}$	μM
7	165.5	386
11	208	442
23	45.6	103
24	25.2	57
25	16.5	41
30	31.6	65
27	30	75
29	25	62

IC₅₀ values μM Figure 2.31: IC₅₀ values of compounds **7**, **11**, **23**, **24**, **25**, **30**, **27** and **29** (μM)

The effect of this set of compounds on the percentage viability of U937 cells was also examined. Again, dihydro derivatives **7** and **11** showed poor biological activity giving ~ 33% and ~ 51% viability at a concentration of 200 $\mu\text{g/mL}$. Both compounds have an aliphatic side chain, and differ only in the functional group, namely alcohol or acetate, at C-3. These

compounds were the poorest in terms of IC₅₀ values also, giving results of 165.5 µg/mL and 208 µg/mL respectively.

Epoxides **23** and **24** showed improving activity giving values of ~ 31% and ~ 8% at concentrations of 100 µg/mL and 50 µg/mL respectively. This result shows that keto epoxide **24** is significantly more active than its analogous C-3 alcohol **23**. We were delighted with this result, as we had specifically targeted the C-3 alcohol and ketone derivatives of the side chain epoxide based on the knowledge from our initial biological screen. By comparing **7** to its C-24,25 epoxide analogue **23**, the effect of the epoxide can be seen clearly in the IC₅₀ value, which is decreased from 386 µM to 103 µM.

Aldehyde **25** also displayed good activity, with ~ 14% viability at 100 µg/mL in the U937 cells, and an IC₅₀ value of 41 µM, the best in this series. However, these results are not mirrored in terms of apoptosis, as almost 500 µM of the compound is required to exhibit a 10 fold increase in apoptotic bodies. Aldehyde **25** and its C-3 ketone analogue **29** show similar biological activity in terms of IC₅₀ values, at 41 µM and 62 µM respectively.

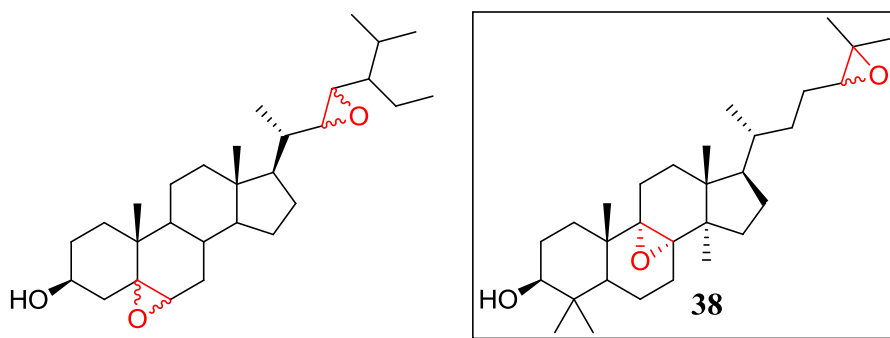
Ketone **30** is quite potent also, giving ~ 7% viability at 50 µg/mL, consistent with its activity in terms of apoptosis, showing a 9 fold increase in apoptotic bodies with 103 µM of the compound. Its IC₅₀ value is moderate at 65 µM. It is worth highlighting the significance of the C-24 ketone in the side chain of **30** as a direct comparison to **11**, as it has a large effect on biological activity of the compounds in U937 cells, with ketone **30** having a ~ 7% viability at 50 µg/mL, while its side chain dihydro analogue **11** shows a viability of ~52% at 200 µg/mL, a four fold increase in concentration.

Compounds **27** and **29** were evaluated at a later date, and in the intermediate time period, we took the decision to report the percentage viability results in terms of µM, to be in line with current literature reporting. Compound **29** was seen to be a more active compound than **27**, as they display ~ 21% and ~ 31% viability respectively. This result is as expected, owing to the extra functionality in compound **29**. Moderate IC₅₀ values of 30 µg/mL and 25 µg/mL are seen for **27** and **29**, while their apoptotic effect is negligible.

2.3.12 Double epoxidation of lanosterol 6

An interesting opportunity arose for the one pot synthesis of the novel diepoxide **38** (mixture of *R* and *S* isomers) with potentially good biological activity. 5,6,22,23-Diepoxystigmastane **39**, formed by the double epoxidation of stigmasterol, had been evaluated against U937 cells in previous work by our collaborators,³⁴ and had displayed promising results (Table 2.7).

Table 2.7 Percentage viable U937 cells following
24 h exposure to **39** at various concentrations

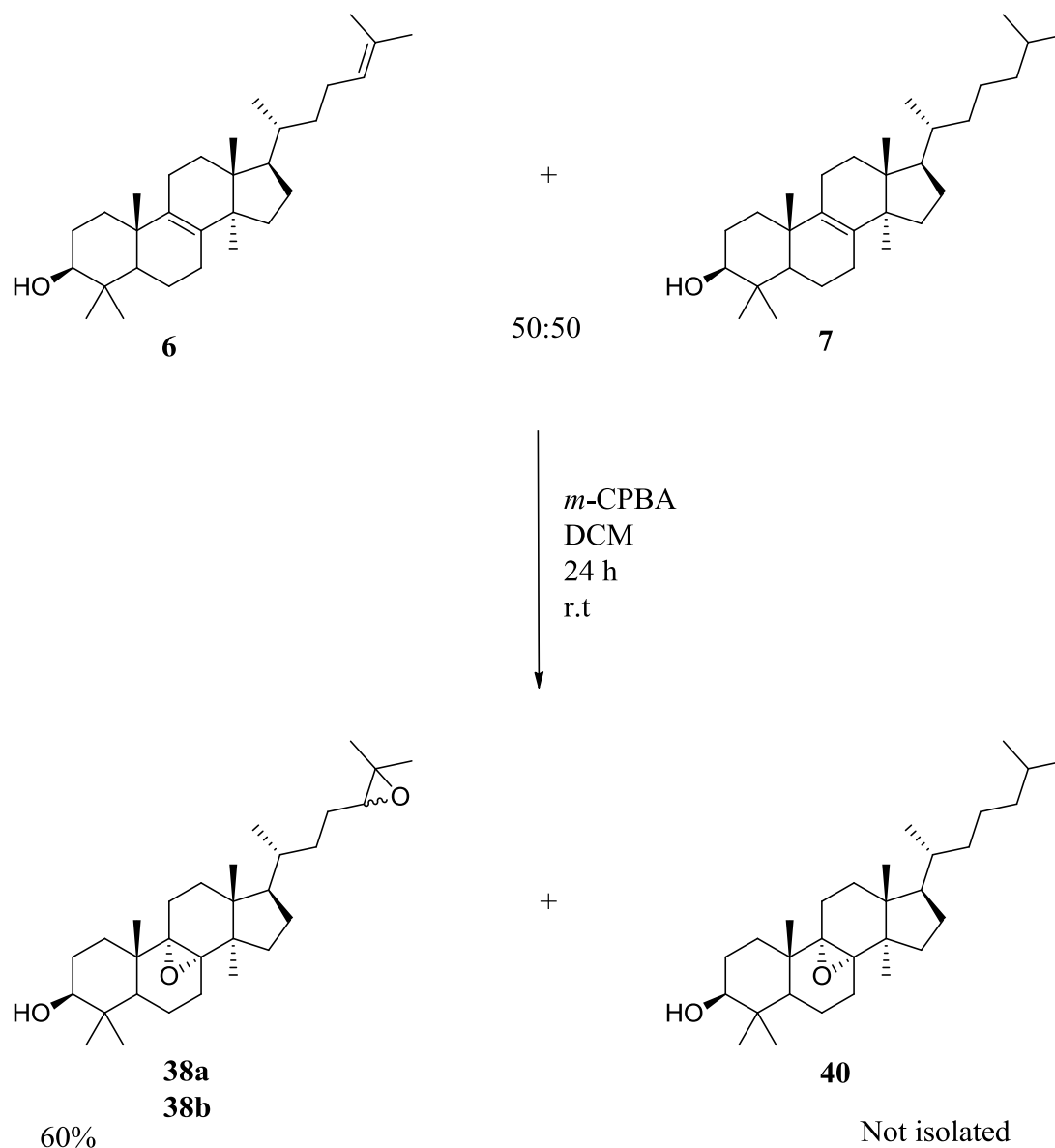


Compound	30 μ M	60 μ M	120 μ M
39	51.4 \pm 8.3	39.6 \pm 4.6	7.6 \pm 2.2

Note: **39** is a mixture of four isomers, α and β epoxides at the C-5,6 position, and *R,R* and *S,S* epoxides at the C-22,23 position.

This compound contains an epoxide in its ring structure at C-5,6 and also in the side chain at C-22,23. Therefore, we saw the potential to make use of the dual alkene functionality of lanosterol, and epoxidise both double bonds available in the ring structure and the side chain.

A mixture of lanosterol **6** and dihydrolanosterol **7** was treated with 3 equivalents of *m*-CPBA to synthesise the novel compound **38** (Scheme 2.32). Separation of the dihydro derivative **40** by column chromatography afforded **38** in 60% yield after repeated chromatography. The side chain epoxide was formed as a mixture of *R* and *S* isomers at the C-24 position, which is consistent with our previous epoxidation work. All literature precedent indicates internal C-8,9 epoxide formation exclusively on the α face.^{9,20}



Scheme 2.32

The side chain epoxide is characterised by the ^1H triplet at 2.68 ppm in the ^1H NMR spectrum. As the transformation from the C-8,9 alkene to the epoxide involves quaternary carbons only, the ^{13}C NMR spectrum is needed as evidence that the C-8,9 epoxide had formed. The new C-8 and C-9 signals are seen at 68.1 ppm and 70.7 ppm respectively in the ^{13}C NMR spectrum.

This novel compound was fully characterised and prepared for biological testing. It was hoped that this compound would be biologically active against the U937 cells, given the success of our epoxides in previous screens.

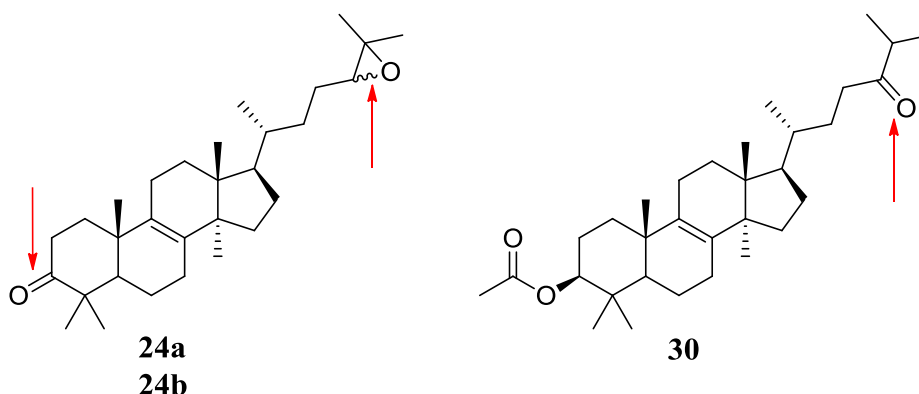
2.3.13 Summary of Lanosterol Oxidation Products

In this area of the project, the research had been developed from previous work to include side chain functionalisation in lanostane derivatives and several interesting and biologically active compounds have been synthesised.

Several structural features of *Antrodia camphorata*, such as the C-7,11 conjugated diketone, the C-7,8 and C-9,11 diene and the side chain functionality, have been successfully incorporated into the lanostane steroid, achieving a target established at the outset of the project.

Fourteen of these lanosterol oxidation products underwent biological evaluation, and the results obtained were used to strategically pinpoint future transformations in order to increase biological activity.

Thus far, our most active compounds in terms of apoptosis, IC_{50} value and percentage viability were **24** and **30**, which shows that incorporation of ketone and epoxide groups results in higher cytotoxicity.



2.4 Amine Derivatives of Lanosterol

2.4.1 Solanidine

Solanidine is the aglycone unit of the glycoalkaloid α -solanine, which has been shown to be biologically active in humans, inhibiting acetylcholinesterase.³⁵ Our interest in this compound stemmed from a collaboration within our research group, concerning the use of potato peel as a source of phytopharmaceuticals. Due to difficulties with the isolation of solanidine from the potato peel, and thus the availability of solanidine as a viable precursor, progress with synthetic modification was severely limited, compounded by the fact that commercial solanidine is very expensive.

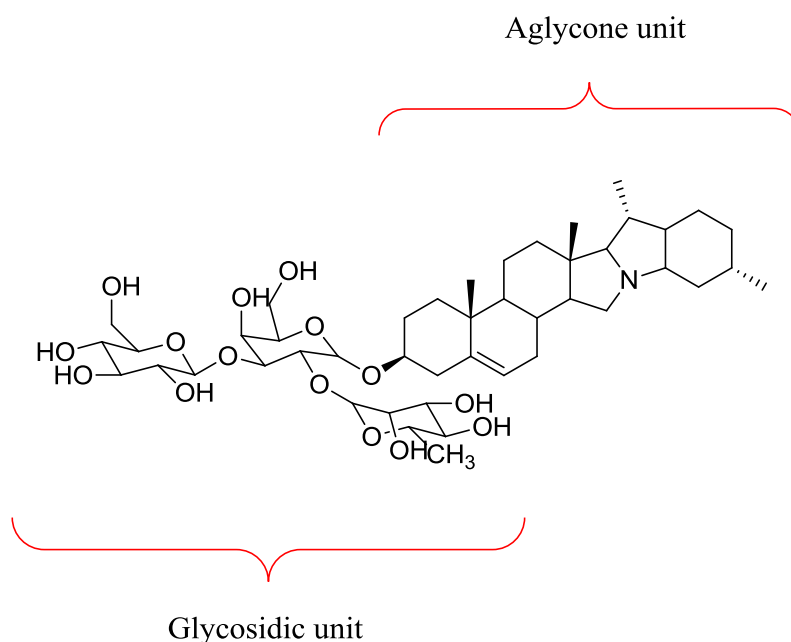
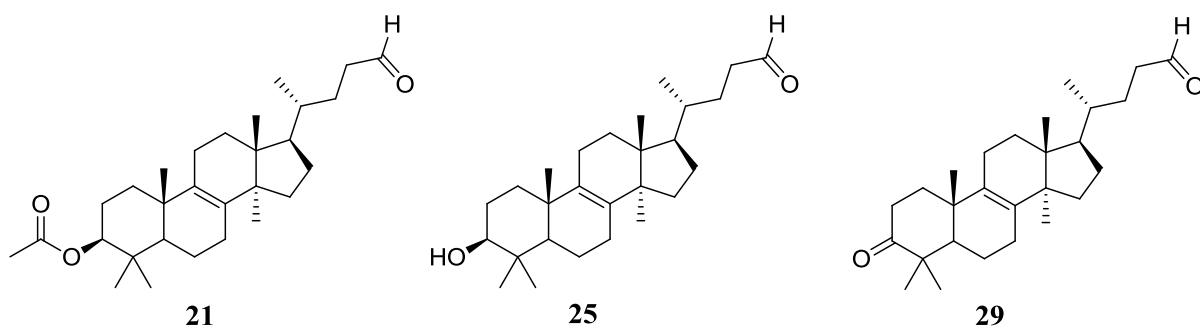


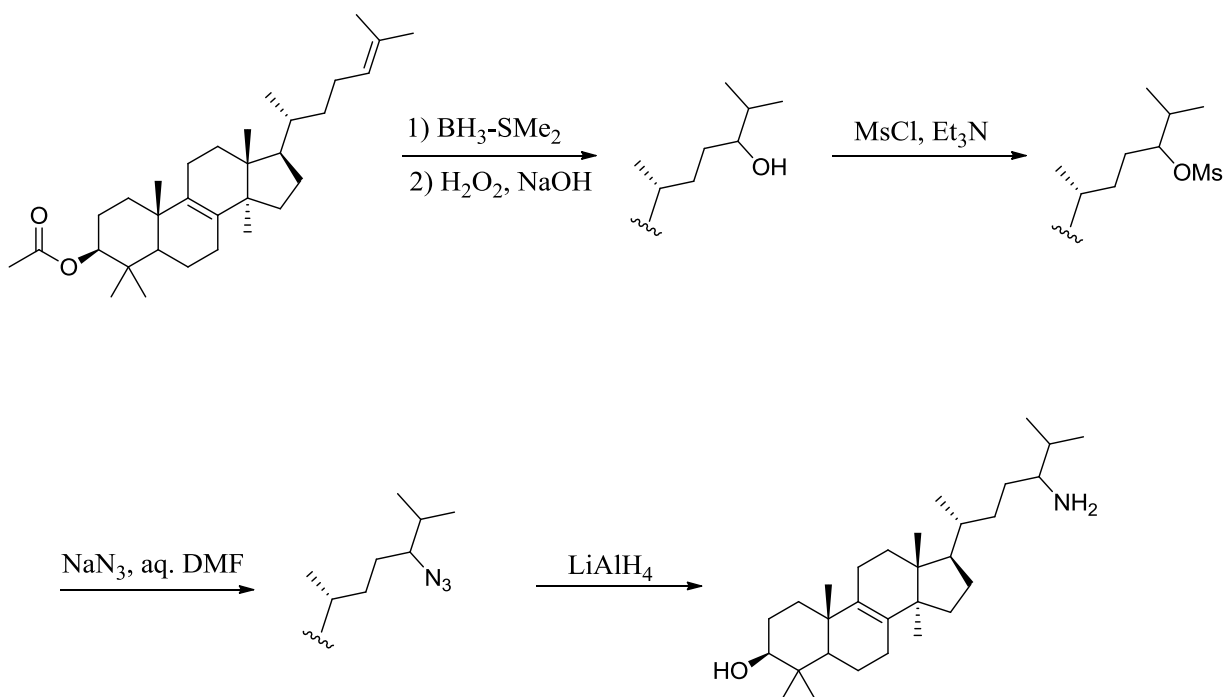
Figure 2.32 α Solanine

Therefore, at this stage in the project our attention turned towards the incorporation of tertiary nitrogen functionality in the side chain of the lanosterol derivatives to mimic the structure and chemistry of solanidine, and to evaluate and compare the biological activity with that of solanidine. Thus, the C-24 aldehyde in compounds **21**, **25** and **29** became crucial in our project because of its potential to undergo reductive aminations.

Figure 2.33 Aldehydes **21**, **25** and **29**

Through a number of test reactions, we successfully established a very efficient one pot reductive amination procedure for the synthesis of amines from the aldehyde derivatives of lanosterol.

While amine derivatives of the cholestane nucleus have been synthesised,^{36,37} there has been limited work carried out on amination of lanostane derivatives. Chung *et al.* have described the synthesis and antifungal activity of a variety of amine derivatives of lanosterol at the C-24 position (Scheme 2.33).⁶ The antifungal activities of these amines against *Cryptococcus neoformans*, a fungal strain considered to be important in connection with AIDS, was seen to improve along the order of ethyl, cyclohexyl, benzyl, methyl and primary amino derivatives. However, a drawback to this work was that amination at this position required multistep synthesis.



Scheme 2.33

Our reductive amination procedure would lead to the synthesis of a variety of novel amines in a one pot reaction from the C-24 aldehyde compound. This reaction also has several advantages over the route described above (Scheme 2.33), namely the elimination of activating groups and of the toxic sodium azide reagent, and scope for a wider variety of amines to be synthesised. Furthermore, our amines would be screened for anticancer activity, which, to the best of our knowledge, has not been reported in the literature for such lanostane derivatives.

2.4.2 Reductive aminations of 3 β -acetoxy-5 α -lanost-8-ene-24-al 21 and 3 β -hydroxy-5 α -lanost-8-ene-24-al 25

Reductive amination is a well known reaction in organic synthesis, whereby aldehydes or ketones react with ammonia, primary or secondary amines in the presence of a reducing agent to yield primary, secondary or tertiary amines respectively. These reactions are considered to be the most efficient carbon – nitrogen bond forming reactions, and an important method for the synthesis of a large scope of amines.^{38,39}

An ordered sequence of steps defines the reductive amination pathway. Firstly, a species known as a carbinol amine is formed, which dehydrates to form an imine. The reaction conditions employed are generally weakly acidic, which allows the imine to be protonated to form an iminium ion. The iminium ion then undergoes reduction to form the alkylated amine product (Figure 2.34).⁴⁰ It is critical to the success of the reaction that a very selective reducing agent is chosen. This is to avoid the unwanted reduction of aldehydes or ketones, and the selective reduction of the iminium ion.

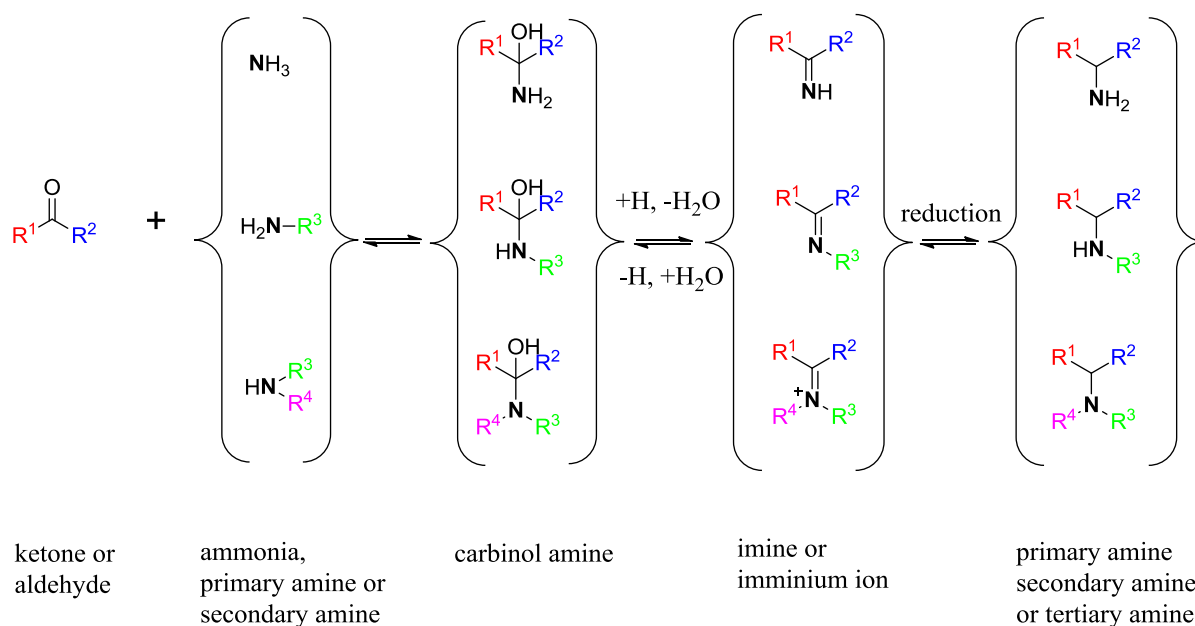
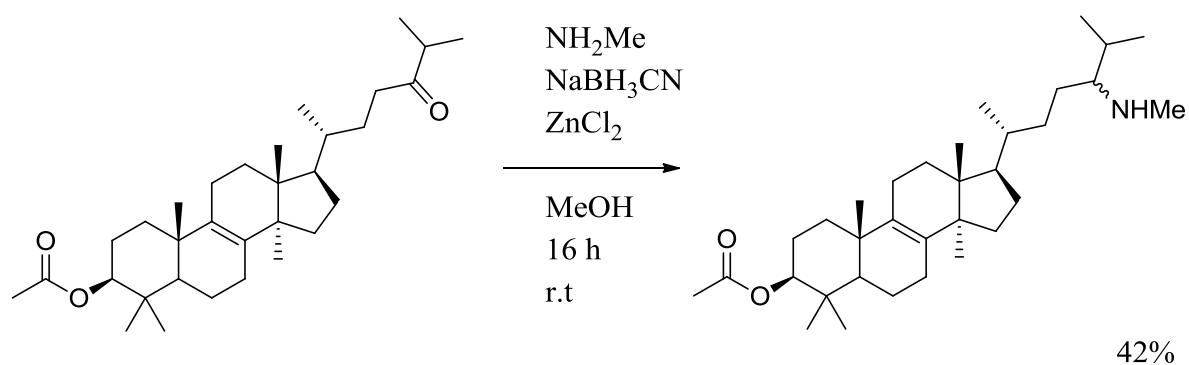


Figure 2.34

Reductive aminations can be described as direct or indirect reactions. The direct pathway refers to when the carbonyl compound and the amine are mixed together with the correct reducing agent, without prior formation of the iminium ion. The indirect (or stepwise) pathway pertains to the pre-formation of the imine or iminium ion followed by reduction in a separate step.

Chung *et al.* report a reductive amination on a C-24 ketone involving the use of sodium cyanoborohydride and zinc chloride (Scheme 2.34).^{6,40}

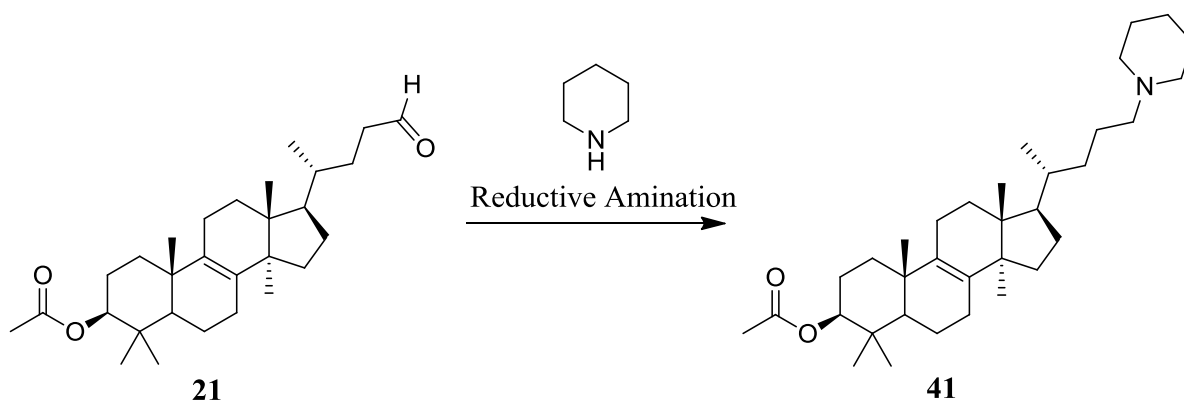


Scheme 2.34

The success of sodium cyanoborohydride as a reducing agent is attributable to its stability in strongly acidic solutions (up to pH 3), selectivity at different pH values, and solubility in hydroxylic solvents.

Our method development was carried out with aldehyde **21** and piperidine (Table 2.8). The reducing agent was used in conjunction with zinc chloride, and stirred in methanol at room temperature for 16 hours,⁶ giving a crude yield of 84%. Attempted column chromatography, using 90:10 hexane: ethyl acetate, to remove minor impurities from the crude material resulted in loss of the product. While successful product formation was observed in the ¹H NMR spectrum of the crude material, this method had its drawbacks. It is known that the reaction may require up to a fivefold excess of the amine, which, while not observed in this case, may have led to complications in future syntheses.⁴⁰ Nonetheless, this method was not ideal, because of potential hazardous by-products and the potential for sluggish reactions with weakly basic amines. Though the 84% yield was satisfactory, the reaction time of 16 hours was quite long, thus we sought a method with shorter reaction time and alternative reagents.

Table 2.8



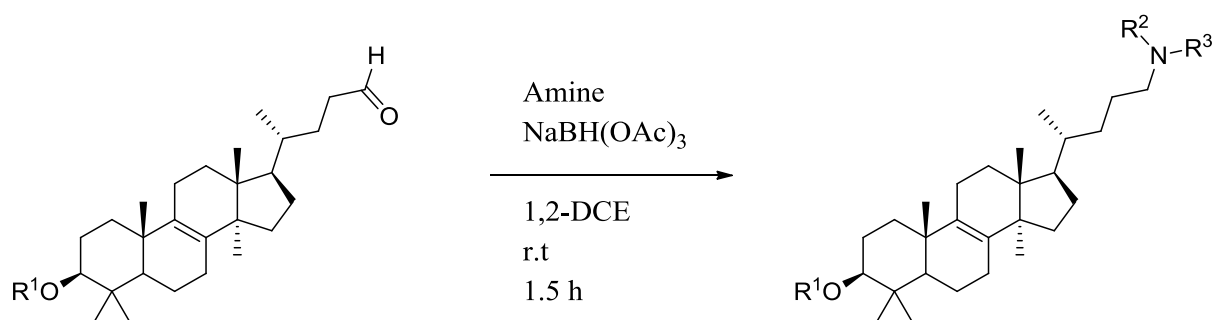
Amine	Reducing Agent	Solvent	Temperature	Time	Crude Yield
Piperidine	NaBH ₃ CN	MeOH	r.t	16 h	84%
	ZnCl ₂				
Piperidine	NaBH(OAc) ₃	1,2-DCE	r.t	1.5 h	91%

A literature search then revealed an alternative method for the reductive amination.⁴⁰ The method involved addition of the significantly milder reducing agent sodium

triacetoxyborohydride to a stirring solution of aldehyde and amine in 1,2-dichloroethane. The reducing agent exhibits remarkable selectivity, as no reduction of the aldehyde to form the alcohol at C-24 is observed. The steric and electron-withdrawing effects of the three acetoxy groups stabilise the boron-hydrogen bond resulting in the mild reducing properties of the substance. The reaction was also carried out at room temperature, but with the significantly shorter reaction time of 1.5 hours and a higher yield of 91%. It is evident that this method is superior to that previously reported, and it was employed in future reactions.

We then set about the synthesis of a library of novel lanosterol derivatives which incorporate amines in the side chain. Amines chosen for the first set of reductive aminations were piperidine, diethylamine, morpholine, aniline and thiomorpholine (Table 2.9).

Table 2.9



Entry	Amine	Product	$\text{R}^1=\text{Ac}$ (a)		$\text{R}^1=\text{H}$ (b)	
			Compound	Yield	Compound	Yield
1			41	94%	42	85%
2			43	65%	44	75%
3			45	54%	46	87%
4			47	94%	48	70%
5			49	71%	50	96%

2.4.2.1 Reductive aminations of aldehydes **21** and **25** with piperidine

Aldehyde **21** underwent reductive amination with piperidine to give **41** in 91% yield (Table 2.9, Entry **1a**). The disappearance of the 24-CH aldehyde signal at 9.77 ppm in the ^1H NMR spectrum showed the aldehyde had reacted fully. A new multiplet appeared at 2.25 – 2.30 ppm, which was assigned as 24-CH₂ (adjacent to N). Another new signal at 2.41 ppm, appearing as a 4H broad singlet was assigned to the two CH₂ groups adjacent to the nitrogen of the piperidine ring. The ^{13}C NMR spectrum of the product showed further evidence for successful reductive amination, with three new signals being observed. Signals at 24.4 ppm, 25.8 ppm and 54.5 ppm correspond to piperidine CH₂, piperidine CH₂ \times 2, piperidine N-CH₂ \times 2 respectively. A shift in the C-24 signal from 203.3 ppm in **21** to 59.9 ppm in **41** is the characteristic difference between the starting material and product, therefore the most significant new signal in the ^{13}C NMR spectrum is that at 59.9 ppm. The signals being assigned as two carbons were twice the relative intensity of the other signals in the spectrum (Figure 2.35).

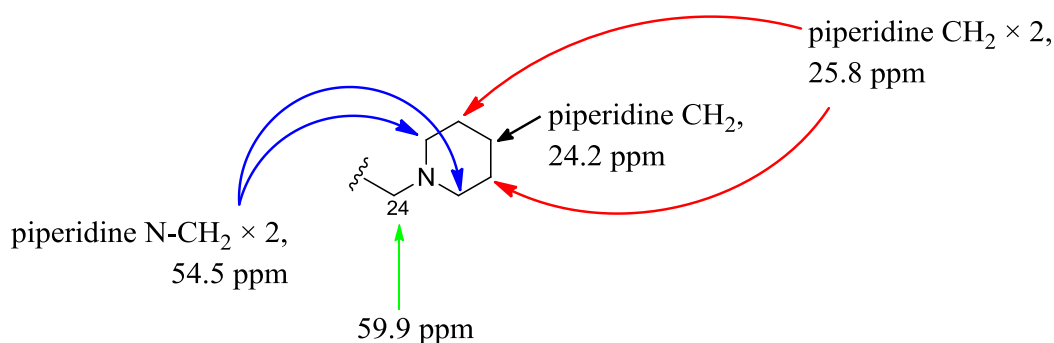


Figure 2.35: Noteworthy ^{13}C NMR data for **41**

Alcohol derivative **25** underwent reductive amination to form **42** in 85% yield (Table 2.29, Entry **1b**). Identical reaction conditions of temperature and time were employed, and no difficulties were observed when the substrate was changed from a C-3 acetate to the more acidic C-3 alcohol. A similar pattern of signals to that described above for the acetate was seen in the ^1H and ^{13}C NMR spectra.

As both of these novel compounds **41** and **42** were synthesised in high purity, they were fully characterised and prepared for biological evaluation.

2.4.2.2 Reductive aminations of aldehydes **21** and **25** with diethylamine

Diethylamine was the next amine to be used in reductive amination reactions. This amine would also yield a tertiary nitrogen at the site of reductive amination. However, there is a difference in the conformational mobility of the diethylamine moiety, when compared to the piperidine moiety. Free rotation of the two ethyl chains may give a difference in reactivity of the amine towards reductive amination, and also biological activity of the derivatives synthesised, due to increased steric hinderance. This may lead to an interesting comparison between the piperidine and diethylamine derivatives in terms of structure activity relationship (Figure 2.36).

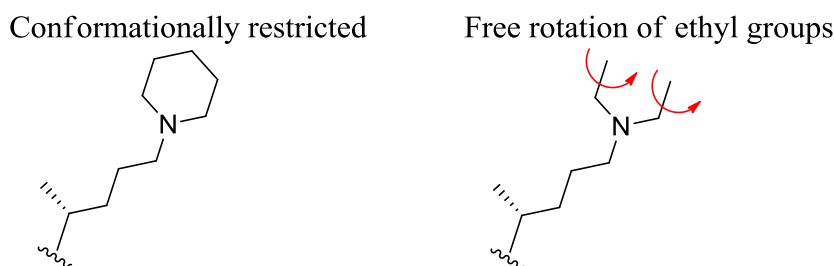


Figure 2.36

Thus, aldehyde **21** was reacted with diethylamine to afford **43** in 65% yield (Table 2.9, Entry **2a**). As anticipated, this yield is ~ 30% less for the diethylamine derivative than for the piperidine derivative. This decrease in yield is possibly due to the free rotation of the ethyl groups hindering reductive amination by restricting the accessibility of the nitrogen lone pair to the carbonyl group. Though a decrease in yield from 94% to 65% is observed, **43** was formed in reasonable yield and high purity, with a sufficient quantity available for biological testing after full characterisation of this novel compound. Evidence for the successful formation of the product can be seen from the ^1H and ^{13}C NMR spectra. The ^1H NMR spectrum shows complete loss of the 24-CH aldehyde proton of the starting material, given that there is no signal at 9.77 ppm. Two overlapping 3H triplets at 1.06 ppm, appearing as a

6H triplet, are assigned to the diethylamine $\text{CH}_3 \times 2$. The 24- CH_2 multiplet appears in the usual region of 2.32 – 2.51 ppm, while the 4H multiplet at 2.52 – 2.67 ppm represents the diethylamine $\text{CH}_2 \times 2$. The ^{13}C NMR spectrum of the product is also quite indicative of successful reductive amination. The strong signals at 11.2 ppm and 46.6 ppm represent diethylamine $\text{CH}_3 \times 2$ and $\text{N-CH}_2 \times 2$. 24- CH_2 remains in the same region as the piperidine derivatives, in this case having a signal at 53.2 ppm (Figure 2.37).

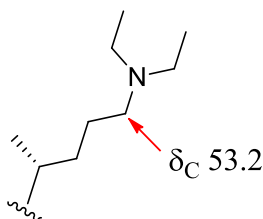


Figure 2.37: Noteworthy ^{13}C NMR signal for **43**

The aldehyde bearing an alcohol at C-3 **25** also underwent reductive amination with diethylamine. In this case product **44** was formed in 75% yield (Table 2.9, Entry **2b**). It is interesting to note that, in this case, there was a 10% decrease in the yield of **44**, as compared with its analogous piperidine derivative **42**. This is an improvement on the ~30% decrease in yield seen between acetate derivatives **41** and **43** (Table 2.9, Entries **1a**, **1b**, **2a**, **2b**). The main conclusion drawn from this section of Table 2.9 is that piperidine is the more reactive amine in reductive amination reactions, which we believe is due to the nitrogen lone pair being sterically unhindered. Spectroscopic evidence for the formation of the desired product **44** correlates with the previous spectra for the product of the reductive amination on the acetate derivative **43**. Again, these two novel compounds were fully characterised and prepared for biological screening.

Having had much success with these piperidine and diethylamine reductive aminations, we were interested in incorporating cyclic amines containing heteroatoms. Our first choice of amine was the readily available morpholine, which is a natural analogue of piperidine. The effect of the contrasting electronic properties of the oxygen in morpholine relative to the CH_2 group in piperidine would be interesting to compare in these new derivatives. It is feasible that the hydrogen bond accepting properties of oxygen could lead to a difference in reactivity of morpholine in the reductive amination reactions.

2.4.2.3 Reductive aminations of aldehydes **21** and **25** with morpholine

Aldehyde **21** reacted with morpholine to form **45** in 54% yield (Table 2.9, Entry **3a**). This yield was considerably more modest than the previous reactions. However, there was no trace of starting material in the ^1H NMR spectrum of **45**, and it transpired that this moderate yield was a once off occurrence, though it is unclear why the yield in this instance was so low.

The influence of the heteroatom on the ^1H NMR spectrum was significant. A 4H triplet at 3.72 ppm represents the overlapping morpholine $\text{O-CH}_2 \times 2$. This signal was much further downfield than any non-heteroatom bearing amine which was attached before. The broad singlet at 2.44 ppm, representing the morpholine $\text{N-CH}_2 \times 2$, is comparable to the $\text{N-CH}_2 \times 2$ at 2.41 ppm in the piperidine analogue. This shows that the oxygen in morpholine only has an effect on the adjacent CH_2 groups. The ^{13}C NMR spectrum is as expected, with the $\text{N-CH}_2 \times 2$ and C-24-CH_2 signals appearing at 53.8 ppm and 59.7 ppm respectively. The key shift in the C-24 signal from 203.3 ppm in the starting aldehyde to ~ 60 ppm in the reductive amination product is observed again in this case. In correlation with the ^1H NMR spectrum, the morpholine $\text{O-CH}_2 \times 2$ is considerably further downfield, appearing at 67.0 ppm in the ^{13}C NMR spectrum.

Alcohol **25** was also reacted with morpholine in a reductive amination reaction to give **46** in 87% yield (Table 2.9, Entry **3b**). Again, no starting aldehyde was observed in the ^1H NMR spectrum of **46** and almost identical signals are seen in the spectra of the acetate and alcohol analogues of the morpholine derivatives, **45** and **46** (Figure 2.38). The main difference between these sets of spectra is the 3-CH signal, which is at its usual positions, ie. 4.50 ppm in the acetate derivative and 3.23 ppm in the alcohol derivative.

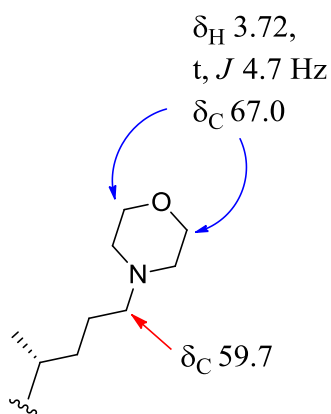


Figure 2.38: Noteworthy signals in ^1H and ^{13}C NMR spectra for both morpholine derivatives **45** and **46**

A striking comparison between the piperidine derivatives, **41** and **42**, and morpholine derivatives, **45** and **46**, (Table 2.9, Entries **1a**, **1b**, **3a** and **3b**) is that a similarly good yield is seen for the C-3 alcohols (85% and 87% respectively). By contrast, the C-3 acetates show a 40% difference in yield, decreasing from 94% in the piperidine derivative to 54% in the morpholine derivative. However, all other acetate derivatives (Table 2.9, Entries **1a**, **2a**, **4a**, and **5a**) display good to excellent yields. Therefore it can be postulated, with reasonable certainty, that the acetate group is not impairing the reaction outcome, but rather this low yield of 54% for compound **45** is a once off occurrence.

2.4.2.4 Reductive aminations of aldehydes **21** and **25** with aniline

Our attention turned towards the incorporation of an aromatic group to vary the side chain derivatives (Table 2.9, Entries **4a** and **4b**). We chose aniline as the aromatic amine because it is a cheap and readily available starting material. It was envisaged that the nitrogen lone pair may have reduced nucleophilicity as it is in conjugation with the aromatic ring. However, in our work, this was not the case and successful reductive amination yielded both alcohol and aldehyde derivatives.

Aldehyde **21** underwent reductive amination with aniline to give product **47** in 94% yield. This yield is an excellent result, both in terms of reactivity and yield, since we envisaged possible reduced nucleophilicity of the lone pair in the amine. The ^1H NMR spectrum of this product is quite different to those previously described. Firstly, the aromatic peaks are evident in the 6.50 – 7.20 ppm region of the spectrum. All five hydrogens of the aromatic ring are

accounted for in this region. The 24-CH₂ signal is shifted slightly downfield, at 3.01 – 3.13 ppm. This methylene group is now adjacent to a free NH, which gives rise to this downfield shift. The ¹³C NMR spectrum shows 24-CH₂ has shifted upfield to 44.6 ppm from 203.3 ppm in the starting aldehyde. This upfield shift is expected, however it is significantly further upfield than was observed for other amine products, which showed the 24-CH₂ signal at ~ 60 ppm (Figure 2.39). This observation can be rationalised by the fact that 24-CH₂ is adjacent to a secondary amine rather than a tertiary amine in this case. The carbons on the aromatic ring are seen in the expected region, at 112.7 ppm, 117.1 ppm, 129.2 ppm and 148.6 ppm.

The ¹H NMR spectrum of the alcohol derivative **48**, synthesised from aldehyde **25**, shows a majority of similarities to the acetate derivative **47**. The main difference between this spectrum and that of **47** is the 3-CH appearing as a doublet of doublets at 3.23 ppm. A similar case is seen in the ¹³C NMR spectrum, where a majority of common signals are seen in both aniline derivatives **47** and **48**.

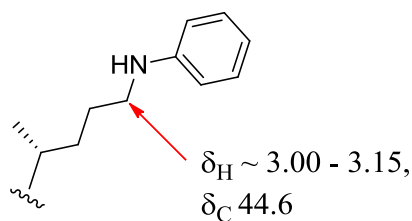


Figure 2.39: Noteworthy signals in ¹H and ¹³C NMR spectra for both aniline derivatives **47** and **48**

A comparison can be drawn between the piperidine and aniline derivatives (Table 2.9, Entries **1a**, **1b**, **4a** and **4b**) in terms of reaction yield. In these cases, the acetate derivatives of both amines gave an excellent yield of 94%, while the alcohol derivatives **1b** and **4b** showed lower yields at 85% and 70% respectively.

2.4.2.5 Reductive aminations of aldehydes **21** and **25** with thiomorpholine

As will be discussed in the biological results of these novel amines, the morpholine derivatives **45** and **46** showed quite poor toxicity in our biological screen. Therefore, we were enthusiastic to explore the biological effects of other heteroatoms in this position, namely sulfur and nitrogen. Thiomorpholine was chosen as the amine for the synthesis of further derivatives.

Aldehyde **21** was reacted with thiomorpholine using our standard reductive amination procedure. The desired product **49** was formed in 71% yield (Table 2.9, Entry **5a**) as an orange solid. We were enthusiastic to see this return to good yield from the 54% displayed by morpholine analogue **45** (Table 2.9, Entry **3a**), reaffirming that the heteroatom does not play a role in reaction yield diminishment.

The four CH₂ groups of the thiomorpholine ring could be seen as a multiplet between 2.60 – 2.80 ppm in the ¹H NMR spectrum. This observation is in contrast to the splitting of the four CH₂ groups in the morpholine ring into two distinct sets of signals, ie. the broad singlet at 2.44 ppm correlating to N-CH₂ × 2, and the triplet at 3.72 ppm corresponding to O-CH₂ × 2.

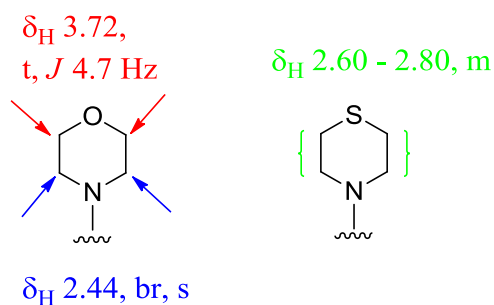


Figure 2.40: Comparison of splitting patterns of morpholine and thiomorpholine derivatives in ¹H NMR spectra

The ¹³C NMR spectrum showed clearer splitting of these four CH₂ signals. The S-CH₂ × 2 could be seen as a strong peak at 28.0 ppm, whereas the N-CH₂ × 2 peak was observed at 55.1 ppm. An interesting comparison of note is that these CH₂ groups adjacent to the sulfur are much further upfield than the analogous morpholine CH₂ groups, which appear at 67.0 ppm in the ¹³C NMR spectrum. As expected, the 24-CH₂ signal appears at 59.9 ppm, in accordance with all other tertiary amines synthesised to date.

The alcohol **25** also underwent reductive amination to form thiomorpholine derivative **50**, as an orange solid in 96% yield, the spectra of which are very comparable to those of **49**. This is the best yield we have obtained for a reductive amination to date, remembering that all of these compounds are synthesised without a need for purification.

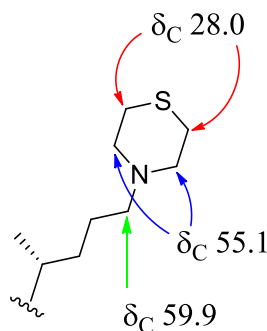


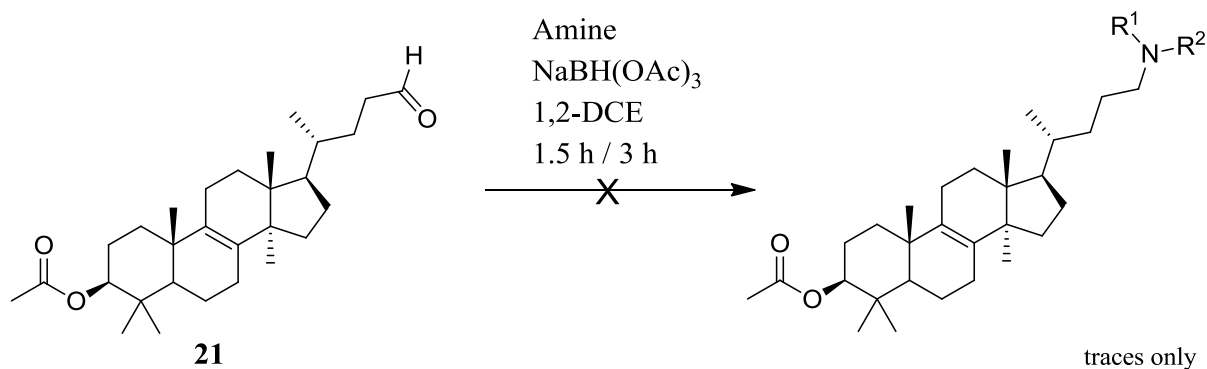
Figure 2.41: Noteworthy signals in ^1H and ^{13}C NMR spectra for thiomorpholine derivatives **49** and **50**

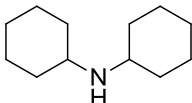
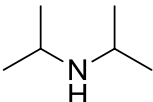
Both novel compounds **49** and **50** were fully characterised and prepared for biological testing.

2.4.2.6 Attempted reductive aminations with dicyclohexylamine, isopropylamine, oxazolidinone and pyrrole

Our next objective was to expand our library using larger amines, while still keeping the tertiary nitrogen in the side chain. However, attempted syntheses of dicyclohexylamine and diisopropylamine analogues (**51** and **52**) were unsuccessful (Table 2.10).

Table 2.10



Entry	Amine	Product	Yield
1		$R^1 = R^2 = \text{cyclohexyl}$ 51	Trace
2		$R^1 = R^2 = \text{isopropyl}$ 52	Trace

Starting material was not fully converted to product.

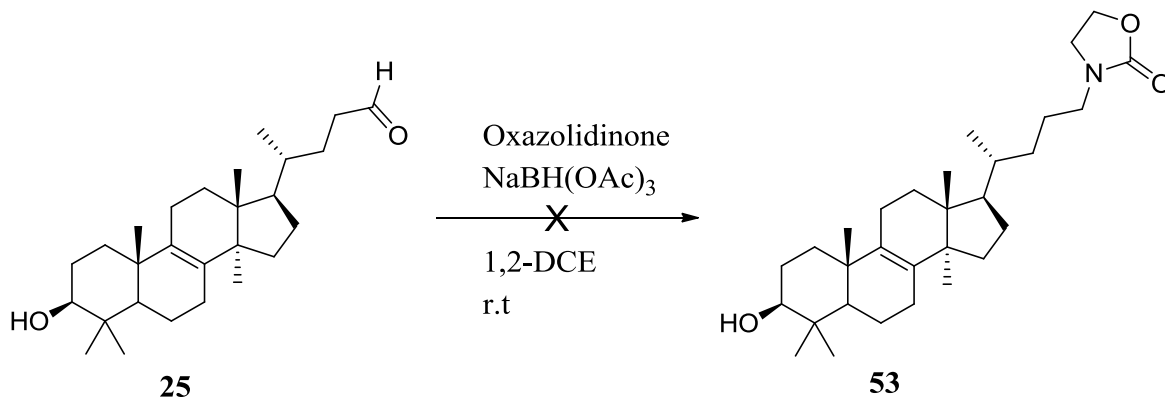
In the case of **51**, after using the standard 1:1 ratio of aldehyde to amine, ^1H NMR analysis revealed the crude product contained 25% aldehyde starting material. When the reaction was repeated using 1.5 equivalents of dicyclohexylamine and an increased reaction time of 3 hours, 14% of the starting aldehyde still remained. This aldehyde was indicated by the signal at 9.77 ppm in the ^1H NMR spectrum, which integrated for 0.14 relative to 1 for the 3-CH signal. This attempt to consume all of the aldehyde was not successful, and created an excess amount of dicyclohexylamine in the product, seen as strong signals at 25.3 ppm, 26.2 ppm, 34.3 ppm, and 53.0 ppm in the ^{13}C NMR spectrum. Our assessment of this reaction was that the nitrogen lone pair of the dicyclohexylamine was too sterically hindered to approach the carbonyl carbon. Given our previous difficulty with column chromatography, when attempting to remove impurities from **41** after the test reductive amination using sodium cyanoborohydride resulted in the loss of all product (Section 2.4.2, page 134), purification was not attempted on this material.

With this new knowledge of the reactivity of sterically bulky amines, we attempted another reaction. This time, the less bulky diisopropylamine was chosen. However, after standard reaction conditions, the ^1H NMR spectrum of the crude product showed 55% starting aldehyde with no evidence of product formation.

Having synthesised several six membered cyclic amine derivatives of lanosterol, we began an initial investigation into reductive amination with a five membered cyclic amine, namely oxazolidinone, to expand on our library of novel compounds.

Aldehyde **25** was reacted with oxazolidinone in an attempt to synthesise **53** (Scheme 2.35). Upon reaction completion and work up, ^1H NMR analysis showed a majority (75%) of

starting aldehyde present. Trace oxazolidinone signals were also observed, including the NH signal, however there was no evidence of successful product formation.



Scheme 2.35

This reaction was repeated using the crude material from the first attempt, with the reaction time increased to 24 h. In this case, the product consisted of 55% starting aldehyde with no increase in oxazolidone signals or any evidence of product formation.

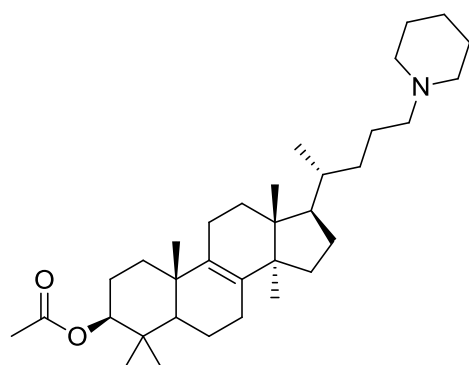
It was unfortunate that this novel lanostane structure with an interesting amine side chain could not be synthesised. Should future work be undertaken in this area, amines with no potential for delocalisation would be favourable.

Given the impurity of **51** and **52**, and the unsuccessful formation of **53**, these compounds were not available for biological evaluation. However, we proceeded with biological screening on the pure amines (Table 2.11). These samples were tested against U937 cells in terms of apoptosis, IC_{50} value and percentage viability of the cells after treatment with the test compounds.

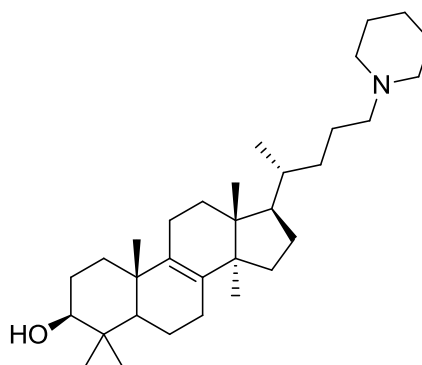
2.4.3 Biological testing 3

The results of this screening were quite promising, and showed a remarkable increase in toxicity at lower concentrations towards the lanosterol oxidation products. The results are presented below.

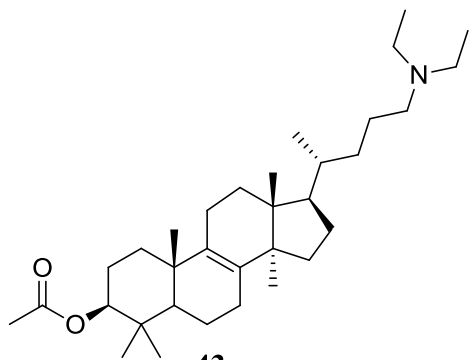
Table 2.11 Biological screening of amine derivatives.*



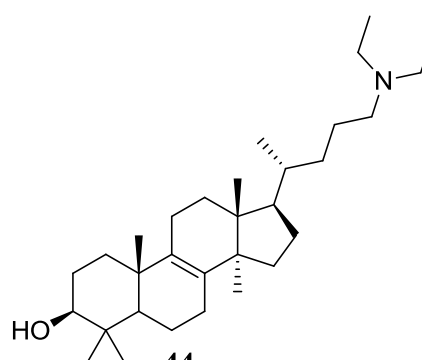
41
Apoptosis = 98 μ M
10.6 fold increase



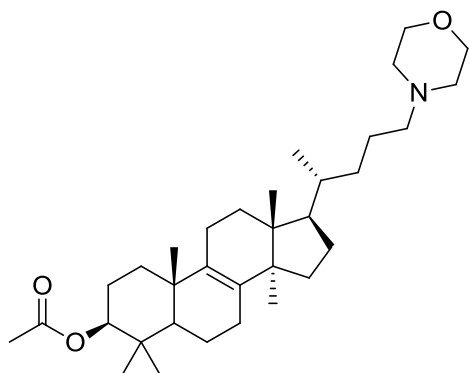
42
Apoptosis = 21 μ M
5.5 fold increase



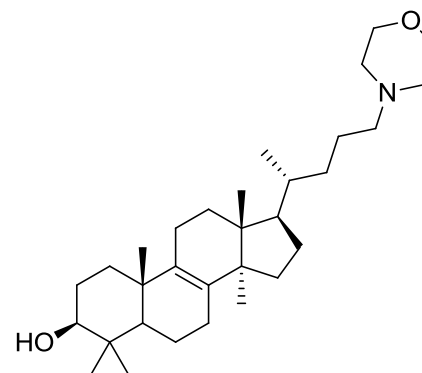
43
Apoptosis = 20 μ M
6.9 fold increase



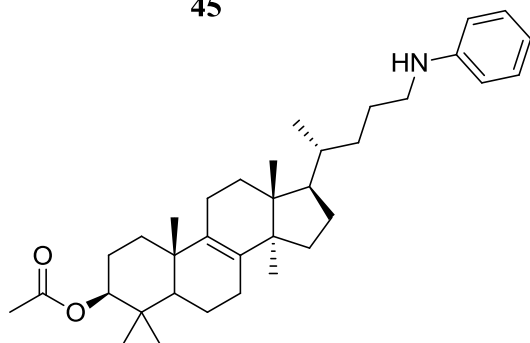
44
Apoptosis = 22 μ M
3 fold increase



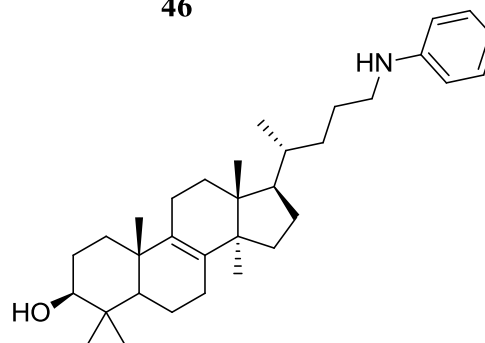
45



46



47



48

% Viability of U937 cells			
	10 $\mu\text{g/mL}$	50 $\mu\text{g/mL}$	200 $\mu\text{g/mL}$
41	41.39 \pm 3.47	27.98 \pm 2.71	-
42	3.92 \pm 0.23	-	-
43	34.86 \pm 8.01	-	-
44	3.73 \pm 0.32	-	-
45	83.96 \pm 3.09	71.45 \pm 3.55	26.91 \pm 0.47
46	92.70 \pm 2.94	71.05 \pm 3.64	5.24 \pm 0.49
47	99.87 \pm 2.90	96.38 \pm 1.73	50.74 \pm 6.47
48	97.65 \pm 2.94	71.74 \pm 2.99	6.12 \pm 0.33

*Thiomorpholine derivatives were not available for this round of biological screening

Percentage Viability of U937 cells at Compound Concentration of 10 $\mu\text{g/mL}$

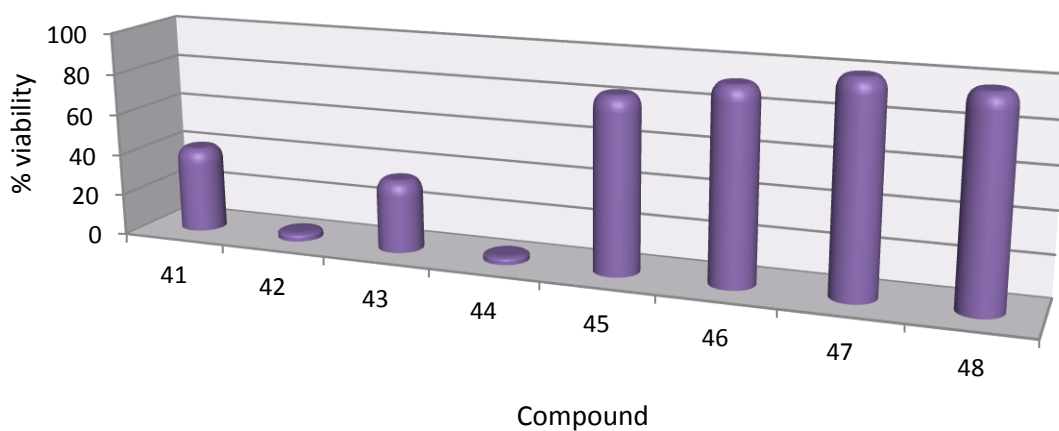
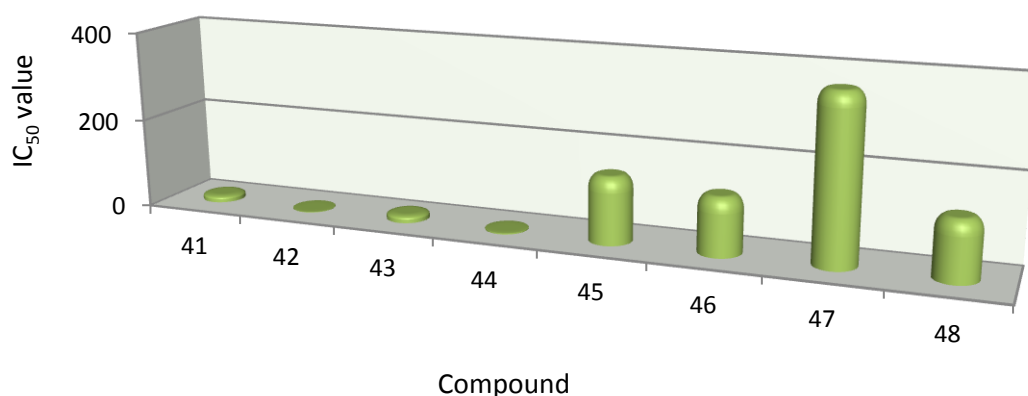


Figure 2.42: Percentage viability of U937 cells following treatment with 10 $\mu\text{g/mL}$ of compounds **41** - **48**.

Table 2.12

	IC ₅₀ values in U937 cells	
	μg/mL	μM
41	7.6	14.9
42	0.9	1.9
43	8.2	16.4
44	1.6	3.5
45	76.1	148
46	60.2	128
47	190.5	367
48	62.8	132

IC₅₀ values in U937 cells μMFigure 2.43: IC₅₀ values of compounds **41** - **48** (μM)

The standout results from this biological screen are from compounds **42** and **44**, giving a percentage viability of U937 cells of ~ 3.9% and ~ 3.7% respectively. This is a marked increase in activity from our previous screen of the lanosterol oxidation products. The concentration of compound is reduced to 10 μg/mL in this case, as compared to ~ 10% viability at 50 μg/mL for epoxide **22**, our best result in the last screen. The IC₅₀ values for these compounds mirror the results expressed in terms of percentage viability of the U937 cells. Compounds **42** and **44** show excellent IC₅₀ values of 0.9 μg/mL (1.9 μM) and 1.6

$\mu\text{g/mL}$ (3.5 μM) respectively, which compares well with etoposide which is a marketed topoisomerase II inhibitor and has an IC_{50} value of 1.5 μM . Thus, we were very happy with these results, as they are our best to date.

Many deductions can be drawn from this new biological information and the knowledge gained would be put to use in future aspects of the project by incorporation of specific functionalities.

Firstly, it is worth noting that the two most active compounds, **42** and **44**, both have alcohols in the C-3 position. Their analogous acetate derivatives, **41** and **43**, gave an approximately 10 fold poorer result at the same concentration, having viabilities of $\sim 41\%$ and $\sim 35\%$ respectively and IC_{50} 's of 7.6 $\mu\text{g/mL}$ and 8.2 $\mu\text{g/mL}$ respectively. The increased biological effect of these alcohols may be due to improved solubility or less steric hinderance imposed by their analogous acetate groups. However, it is very interesting to note, that in terms of apoptosis, a reverse biological effect is observed. At relatively similar concentrations of 20 and 22 μM , **43** exhibited a 6.9 fold increase in apoptotic bodies, whereas **44** showed a 3 fold increase. In this instance, the acetate diethylamine derivative has an improved biological effect as compared to the alcohol diethylamine derivative.

Secondly, a comparison between the piperidine and morpholine derivatives, **42** and **46**, is also striking. On replacement of the CH_2 group by an oxygen atom, the biological activity is completely diminished, going from $\sim 4\%$ to $\sim 93\%$ viability. These percentage viability results in the U937 cells are reflected in the IC_{50} values, going from 0.9 $\mu\text{g/mL}$ for **42** to 60.2 $\mu\text{g/mL}$ for **46**.

It is also worth noting the poor results displayed by the aniline derivatives **47** and **48**. These compounds had almost no effect on the U937 cells, **47** being particularly poor, with $\sim 100\%$ viable U937 cells remaining after treatment with the compound, and an IC_{50} value of 190.5 $\mu\text{g/mL}$. Given this knowledge, aniline would not be used for future reductive aminations in the project.

2.4.4 Reductive Aminations

2.4.4.1 Reductive aminations of aldehydes **21** and **25** with piperazine

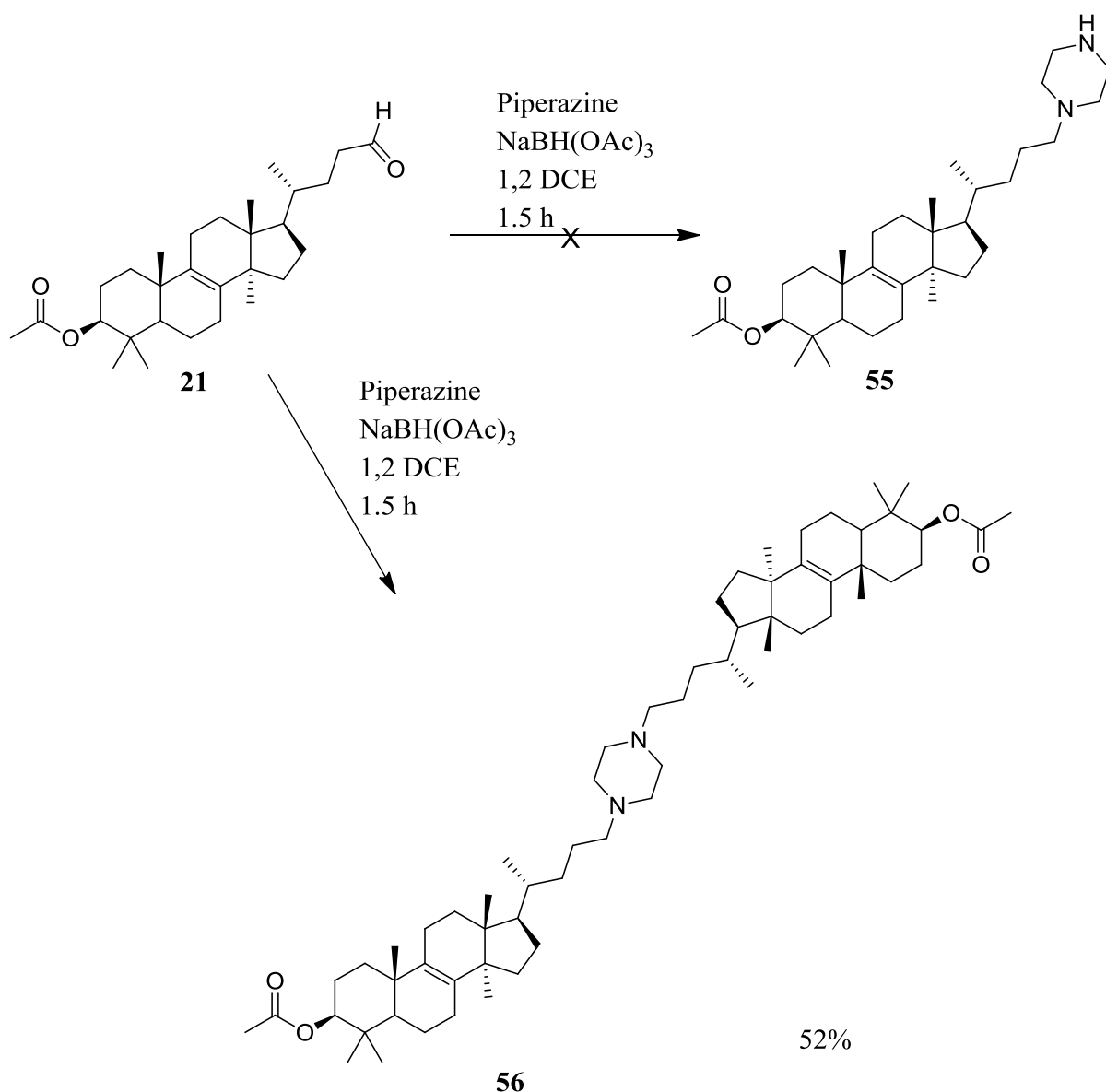
Given the success of the particular amines discussed above, from both a synthetic chemistry and biological activity point of view, we were eager to expand our tertiary nitrogen library of compounds, and introduce the potential for substituted piperazines with longer side chains.

Firstly, we began this area of chemistry with the parent piperazine to ascertain how this amine would behave in our optimised methodology established to date.

Piperazine and aldehyde **21** were reacted together in a reductive amination using sodium triacetoxyborohydride (Scheme 2.37). However, inspection of the ^1H NMR spectrum of the crude material showed a result we had not initially anticipated. We had expected to see a broad singlet at ~ 2.9 ppm in the ^1H NMR spectrum corresponding to the two CH_2 groups adjacent to the free NH in the desired product **55** as reported in the literature, however this signal was not observed.⁴¹ Therefore, it was necessary to explore the possibilities for alternative products formed. We rationalised that, as piperazine contains two nitrogens, both had acted as nucleophiles in the reaction to form a dimer *via* a double reductive amination.

Confirmation of this assignment through ^1H and ^{13}C NMR spectroscopic analysis would prove difficult, as all steroidal signals were overlapping and indistinguishable. However, given that the dimer contains one piperazine ring and two steroidal moieties, it was observed that the integration of the steroidal signals was doubled as compared with the piperazine signals. This NMR evidence is indicative of the presence of a dimer, and high resolution mass spectrometry confirmed our structural assignment. The exact mass calculated for the dimer was 939.7918, and a mass of 939.7879 was found by HRMS, while the mass of the desired product **55**, was not present. With this evidence, we were content to assign the structure of the product as dimer **56**.

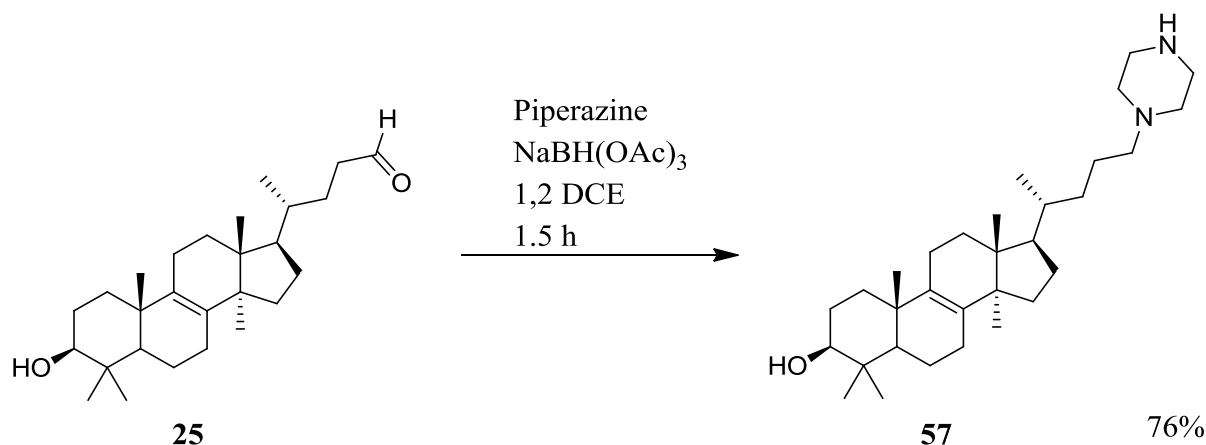
This compound was formed as a white solid in 52% yield. Although the yield is moderate, the compound is formed as a pure dimer, with no evidence of the formation of **55**. Though it was not the intended product of the reaction, it contributed to our novel library of amines and was an interesting synthetic result. The biological activity of this compound would also be evaluated at a later date.



Scheme 2.37

The reductive amination using piperazine was also carried out on aldehyde **25** (Scheme 2.38). As for the acetate, we expected to form a dimer in this case also. However, in this instance, another unexpected result emerged. We were surprised to see a broad singlet at 2.94 ppm in the ^1H NMR spectrum of the product **57**, which corresponds to the two CH_2 groups adjacent to the free NH. This signifies that a dimer had not been formed in this case, but rather, only one of the two nitrogens available had acted as a nucleophile in the reductive amination. The product was submitted for HRMS analysis, which found an exact mass of 471.4309,

correlating closely to the calculated mass of 471.4314 for **57**. The HRMS evidence gives further support to the proposed structure for product **57**.



Scheme 2.38

The formation of the dimer using the acetyl derivative of the aldehyde **21**, and the “monomer” using the alcohol derivative **25**, is a surprising result. This may have been due to increased or decreased solubility of either substrate in the chosen solvent. Electronic effects are unlikely to be the determining factor in this case, as the acetate and alcohol groups are too far removed from the aldehyde moiety to have any influence.

2.4.4.2 Reductive aminations of aldehydes **21**, **25** and **29** with 1-(2-hydroxyethyl)piperazine

As previously discussed, the lanostanes containing the C-3 hydroxyl group was seen to be more biologically active than the C-3 acetate group in our screening to date. Therefore, our interest turned to strategically targeting the synthesis of a molecule with hydroxyl groups at both extremities of the steroid (Figure 2.44). 1-(2-Hydroxyethyl)piperazine was chosen, as it has an OH group at the end of the two carbon chain which could have significant effects on hydrogen bonding and solubility. This amine is also a good choice of starting material because there is no possibility of formation of a dimer in this case, as there is only one free NH in this piperazine derivative.

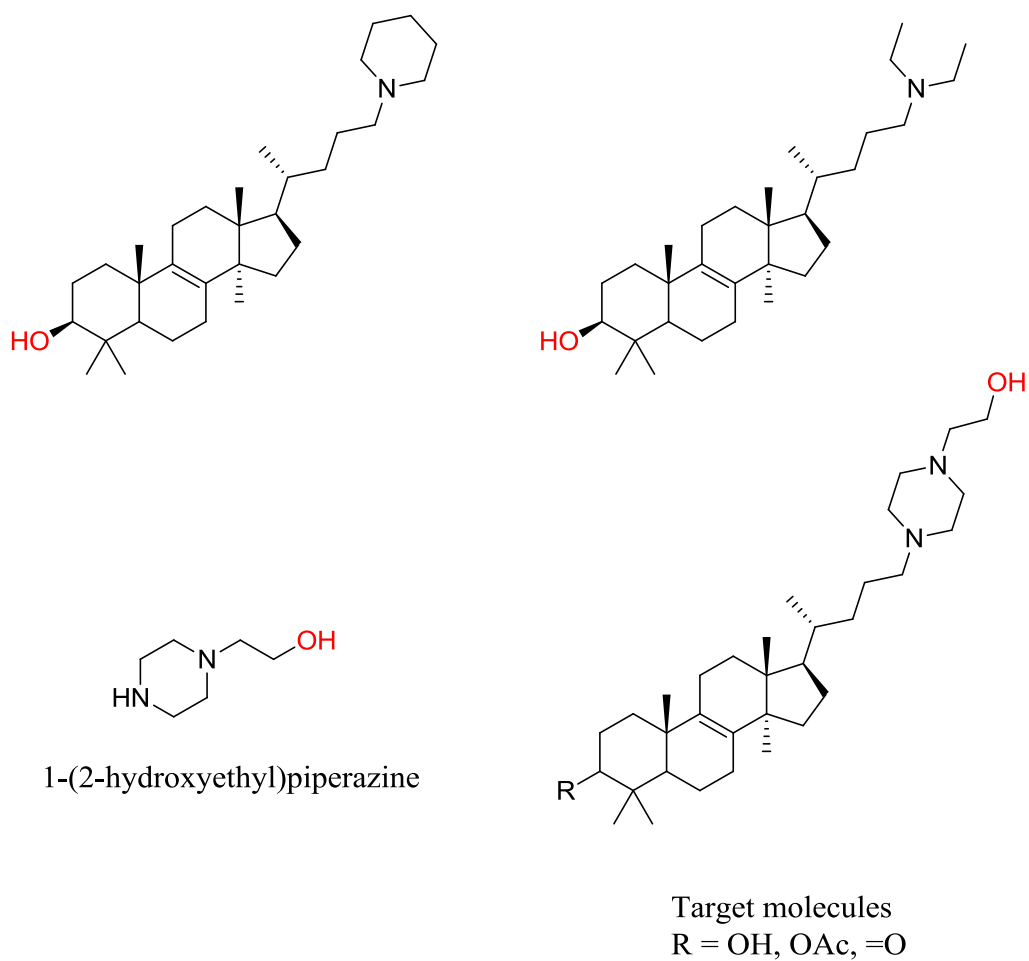
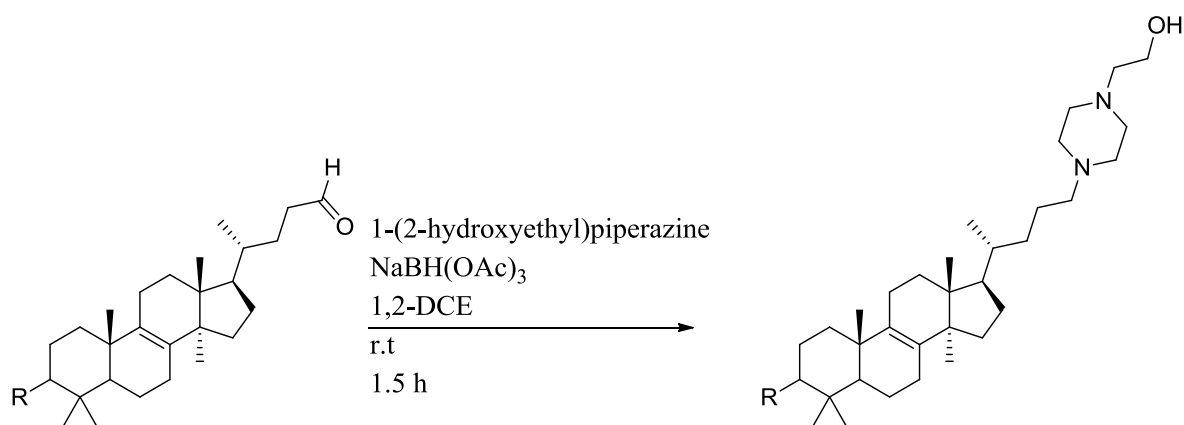


Figure 2.44

The reductive amination of each of the three aldehydes **21**, **25** and **29** with 1-(2-hydroxyethyl)piperazine was carried out under our standard conditions using sodium triacetoxyborohydride as can be seen in Table 2.13. From this point, our series of amines would be expanded to incorporate C-3 acetate, alcohol and ketone functionalities.

Table 2.13



Entry	R	Starting Aldehyde	Yield	Product
1	β OAc	21	94%	58
2	β OH	25	88%	59
3	=O	29	72%	60

Firstly, aldehyde **21** was reacted with 1-(2-hydroxyethyl)piperazine to yield 3 β -acetoxy-5 α -4,4,14-trimethyl-24-piperazino-*N*-2-hydroxyethyl-chol-8-ene **58** as a light orange solid in 94% yield. This excellent yield showed very promising prospects for the use of this amine across the series of C-3 acetate, alcohol and ketone. No difficulties were encountered in terms of reaction workup or solubility when the amine side chain was introduced. However, a minor issue arose in the handling of this amine. As will be discussed in the experimental section, hydroxyethyl piperazine is a very viscous oil, which impairs the ability to measure its mass or volume by conventional methods. Therefore, specific measures were taken to ensure accurate volume determination, as described in due course. Once this issue had been circumvented, no further complications occurred.

Evidence for the formation of the desired product was seen in the ¹H NMR spectrum. Firstly, the absence of the triplet at 9.77 ppm shows that the aldehyde had been entirely consumed and the reaction had gone to completion. Secondly, a new multiplet between 2.24 and 2.37 ppm accounts for the new 24-CH₂ signal. A further multiplet between 2.38 and 2.64 ppm, accounting for 10 hydrogens, corresponds to overlapping signals from piperazine N-CH₂ × 4

and N-CH₂ of the hydroxyethyl moiety (Figure 2.45). A new broad singlet at 3.28 ppm correlates with the presence of the OH group in the new side chain, while the 2H triplet at 3.62 ppm represents the CH₂ group adjacent to the OH.

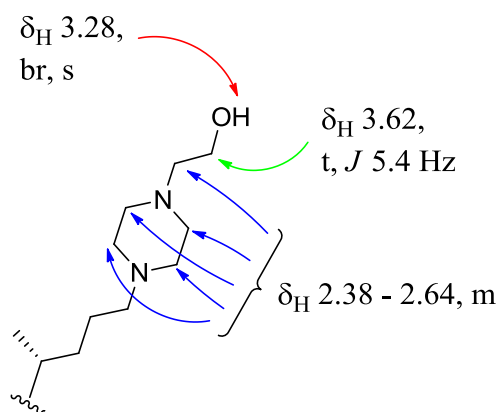


Figure 2.45: ¹H NMR signals of **58**

Aldehyde **25** also underwent reductive amination with 1-(2-hydroxyethyl)piperazine, to yield **59** as an off white solid in 88% yield.

A similar pattern was seen in the ¹H NMR spectrum of this product. The CH₂ group adjacent to the OH in the side chain was seen as a 2H multiplet between 3.52 and 3.66 ppm. In this case, the signal did not appear as a triplet, as was the case for **58**, but rather a multiplet in the same region. The hydroxyl hydrogen, which was clearly visible at 3.28 ppm in the ¹H NMR spectrum of **58**, was not evident in the spectrum of **59**. However, this hydrogen may have been obscured by the nearby doublet of doublets at 3.23 ppm, corresponding to the 3-CH geminal to the C-3 hydroxy group (Figure 2.46).

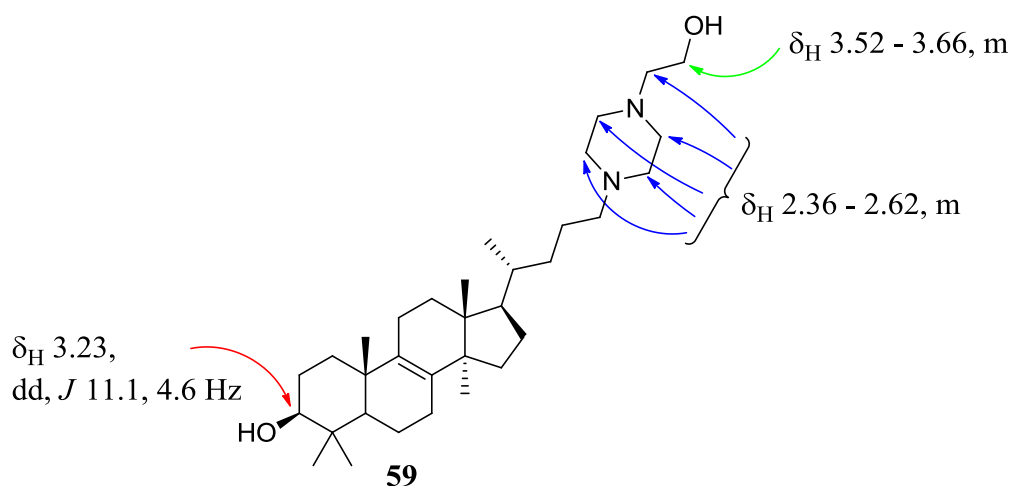
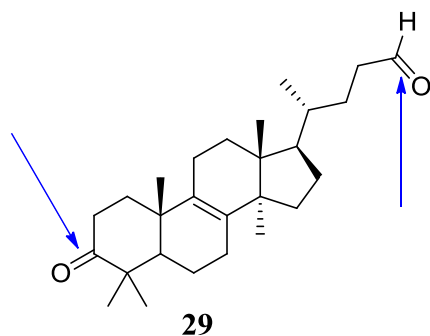


Figure 2.46

At this stage in the project we began to incorporate a C-3 ketone into our series of amine derivatives, with the use of aldehyde **29** in our reductive amination reactions. The aldehyde in this starting material remained at C-24, however C-3 now had a ketone moiety, as opposed to an acetate or alcohol, as in previous cases. There was only a small possibility of reductive amination taking place at the ketone position, given that aldehydes are far more reactive than ketones.

Figure 2.47: Sites of possible reductive amination in starting material **29**

Compound **29** underwent reductive amination with 1-(2-hydroxyethyl)piperazine, to yield **60** as an orange solidifying oil in 72% yield. Though this yield was lower than the previously reported 94% and 88% for acetate and alcohol derivatives respectively, the product was formed in good purity with sufficient quantities available for biological evaluation. The reductive amination reaction proceeded as normal, using our standard conditions of room

temperature and 1.5 hour reaction time, with complete conversion of starting material to product.

Evidence for the formation of this compound is seen in a 12H multiplet between 2.25 and 2.95 ppm in the ^1H NMR spectrum. This multiplet contains piperazine $\text{N-CH}_2 \times 4$ and N-CH_2 of the hydroxyethyl moiety, as well as a 2H triplet at 2.33 ppm, corresponding to 24-CH_2 . As C-3 now bears a ketone moiety, there is no longer a distinct 3-CH signal, as was present in the acetate and alcohol derivatives. Therefore, to establish the presence of the ketone, it is necessary to turn to the ^{13}C NMR spectrum. A quaternary C signal at 218.0 ppm in the ^{13}C NMR spectrum is evidence for the ketone at this position (Figure 2.48).

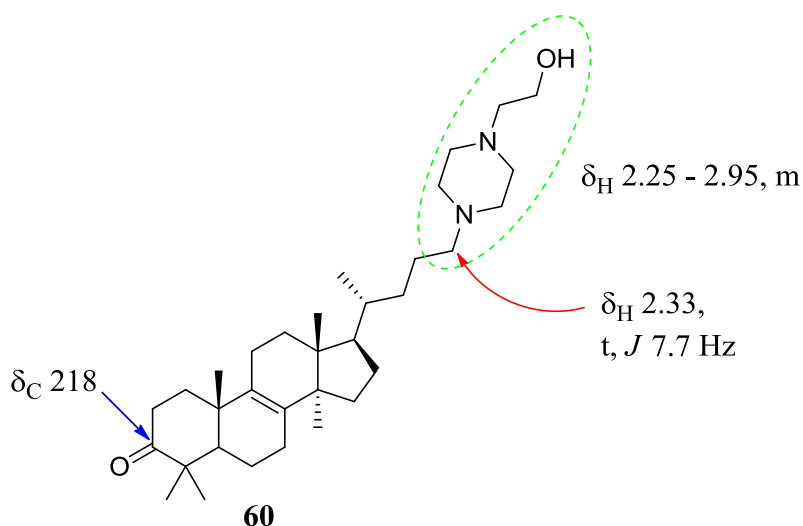


Figure 2.48

A series of novel 1-(2-hydroxyethyl)piperazine derivatives, **58**, **59** and **60**, had now been synthesised and characterised (Figure 2.49). These compounds were synthesised in a sufficiently pure form to be evaluated for biological activity. An interesting comparison between the cytotoxicities of the compounds containing various functionalities at C-3, namely, the acetate, alcohol and ketone, could be established.

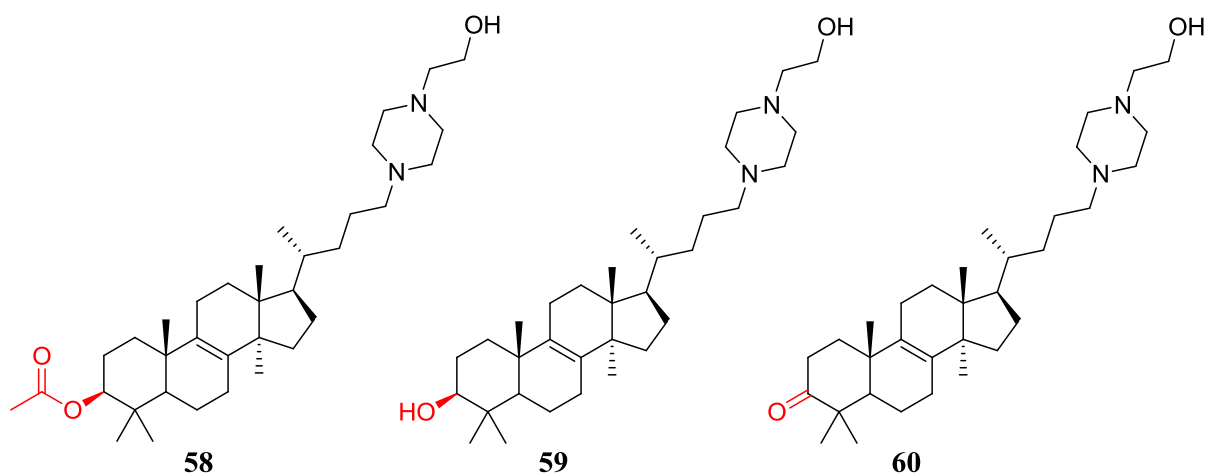


Figure 2.49

2.4.4.3 Reductive Aminations of aldehydes 21, 25 and 29 with 1-(2-aminoethyl)piperazine

Given the success of the reductive amination reactions using 1-(2-hydroxyethyl)piperazine across a series of aldehydes, the next amine chosen for reductive amination reaction was 1-(2-aminoethyl)piperazine (Figure 2.50). This work was carried out in order to synthesise an analogous series to the hydroxyethyl series described above, particularly focusing on a comparison of these compounds in terms of the chemistry and biological activity of the free OH and NH₂ groups.

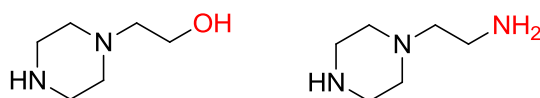
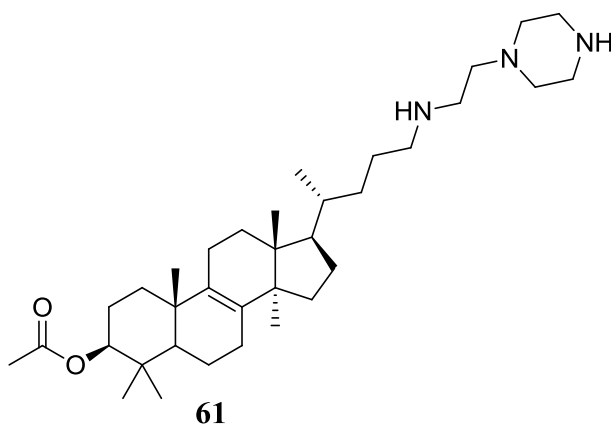


Figure 2.50

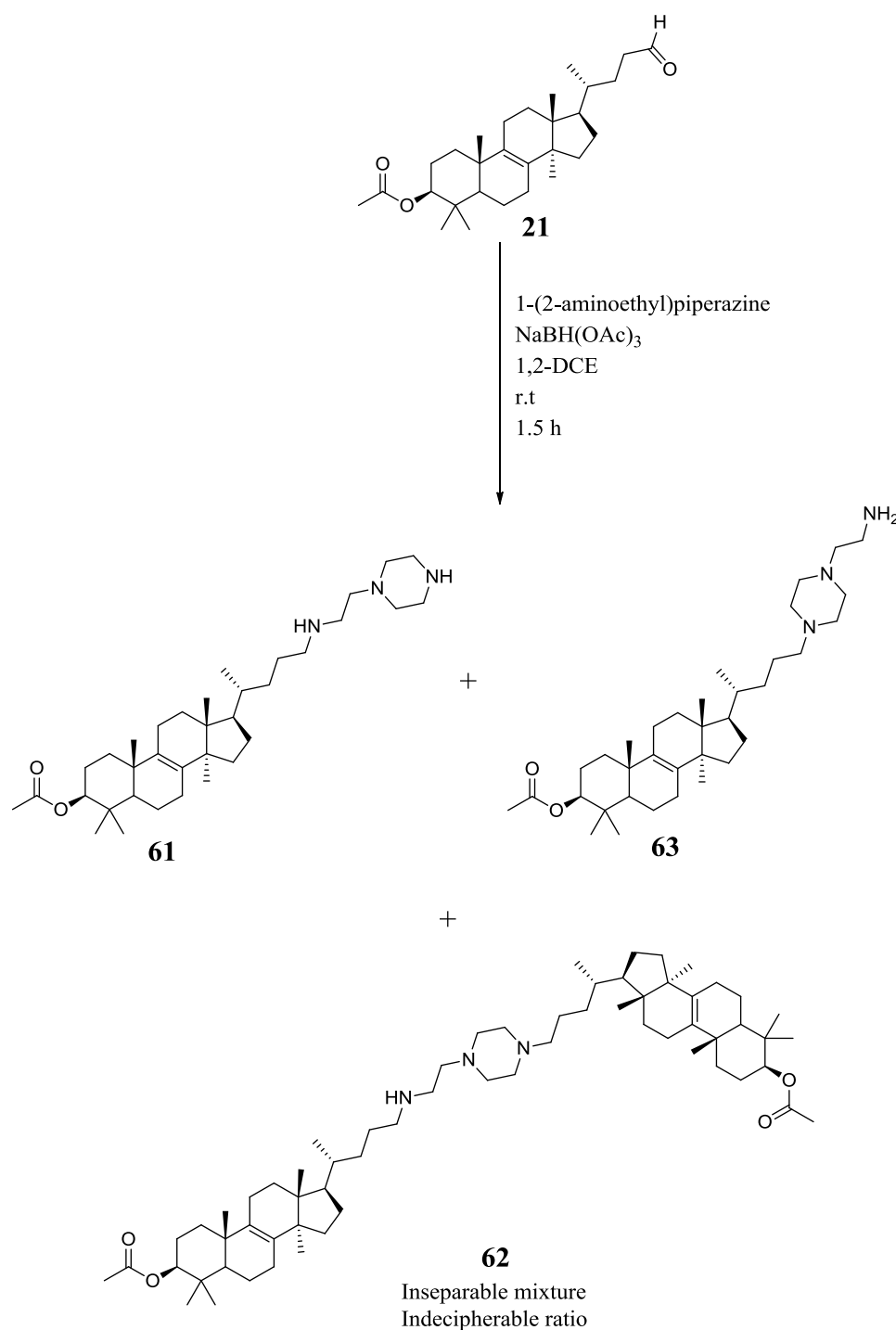
However, prior to carrying out the reaction using 1-(2-aminoethyl)piperazine, we discovered literature evidence that the reaction at the primary amine site is the major pathway in the reductive amination.⁴⁰ Having already purchased this starting material, we proceeded with the reaction, expecting to obtain target molecule **61**. Though the synthesis of this molecule was not our original intention, it was considered to be a useful target in terms of hydrogen bonding and biological activity prospects.

Figure 2.51: Target molecule **61**

Aldehyde **21**, bearing the C-3 acetate group, was the starting material in the reductive aminations with 1-(2-aminoethyl)piperazine. The reaction proceeded using the conditions as described previously, with stirring at room temperature over a 1.5 hour period. However, upon reaction completion and work up, ^1H NMR analysis revealed a complicated mixture of products, and the reductive amination reaction using this amine transpired to be more complicated than first envisaged.

We believe that in addition to obtaining the product from the reductive amination *via* the primary amine pathway, this product subsequently underwent further reductive amination to yield unsymmetrical dimer **62**. In addition, reductive amination *via* the secondary amine pathway was also observed, yielding **63** in trace quantities.

Therefore, we tentatively propose the following three compounds to be present in the crude reaction mixture, see Scheme 2.39 below. Significant overlap of key signals is seen in the ^1H and ^{13}C NMR spectra of all three compounds, rendering an attempt at assigning their relative ratios difficult and inaccurate. Therefore, we are reporting a very tentative assignment of structures in this section for all reductive aminations concerning 1-(2-aminoethyl)piperazine.



Scheme 2.39

Key signals of the aminoethyl side chain can be seen below (Figure 2.52). The peak at ~ 45 ppm corresponds to the CH₂ groups adjacent to the free NH of the aminoethyl ring in regioisomer **61** and is a distinguishing signal for this compound. The peak at ~ 38 ppm is characteristic of regioisomer **63**, and corresponds to the CH₂ group adjacent to the free NH₂ of the aminoethyl side chain. Peaks at ~53 ppm and ~59 ppm are common to both regioisomers, as shown.

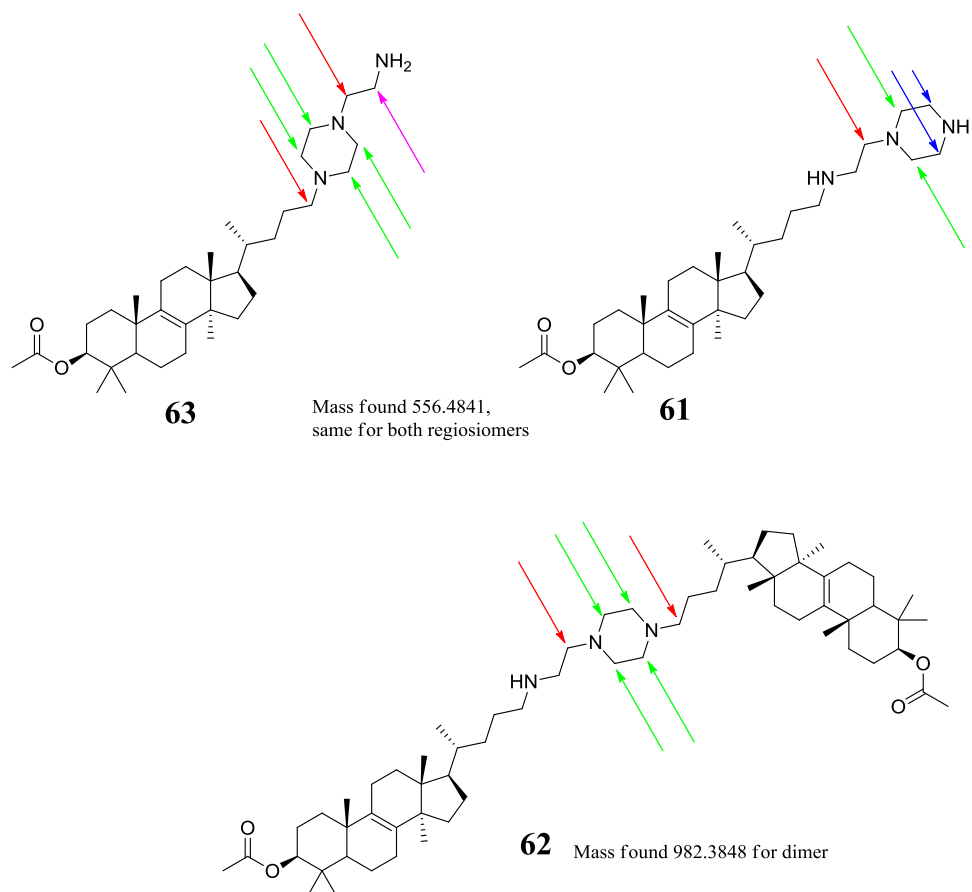
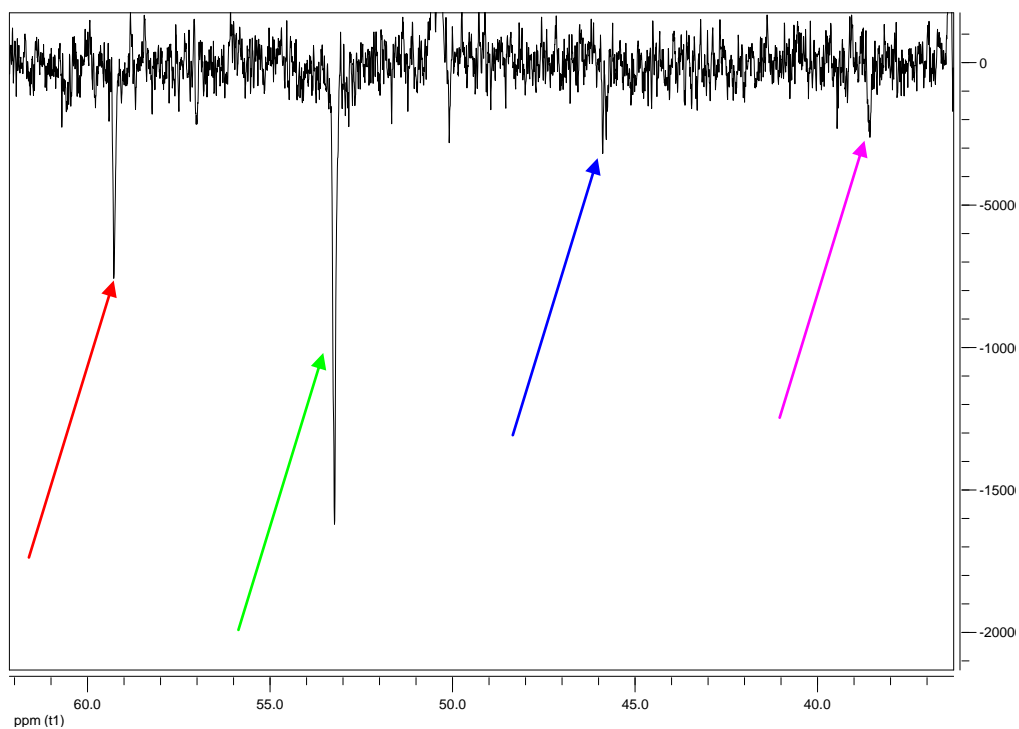


Figure 2.52: ^{13}C NMR spectrum of the mixture of **61**, **62** and **63**, run in the DEPT 135 mode

Evidence for the formation of the dimer lies in the high resolution mass spectrum, where a peak at 982.3848 is seen, corresponding to the exact mass of unsymmetrical dimer **62**. The mass of regioisomers **61** and **63** is also seen at 556.4841 which corresponds to both regioisomers.

By interpretation of the ^1H NMR spectrum, the most upfield multiplet, between 2.16 and 2.26 ppm, is assigned as 24- CH_2 , in accordance with its position in the other amines synthesised to date. However, the signal for this CH_2 group, adjacent to a tertiary amine on one side and another CH_2 group on the other side, could feasibly correspond to either of the other two $\text{N}-\text{CH}_2$ groups shown in Figure 2.53. The signal at 2.90 ppm is due to the two CH_2 groups adjacent to the free NH in the piperazine ring for regioisomer **61**, however the lack of separate distinct signals for each regioisomer renders their ratio indecipherable.

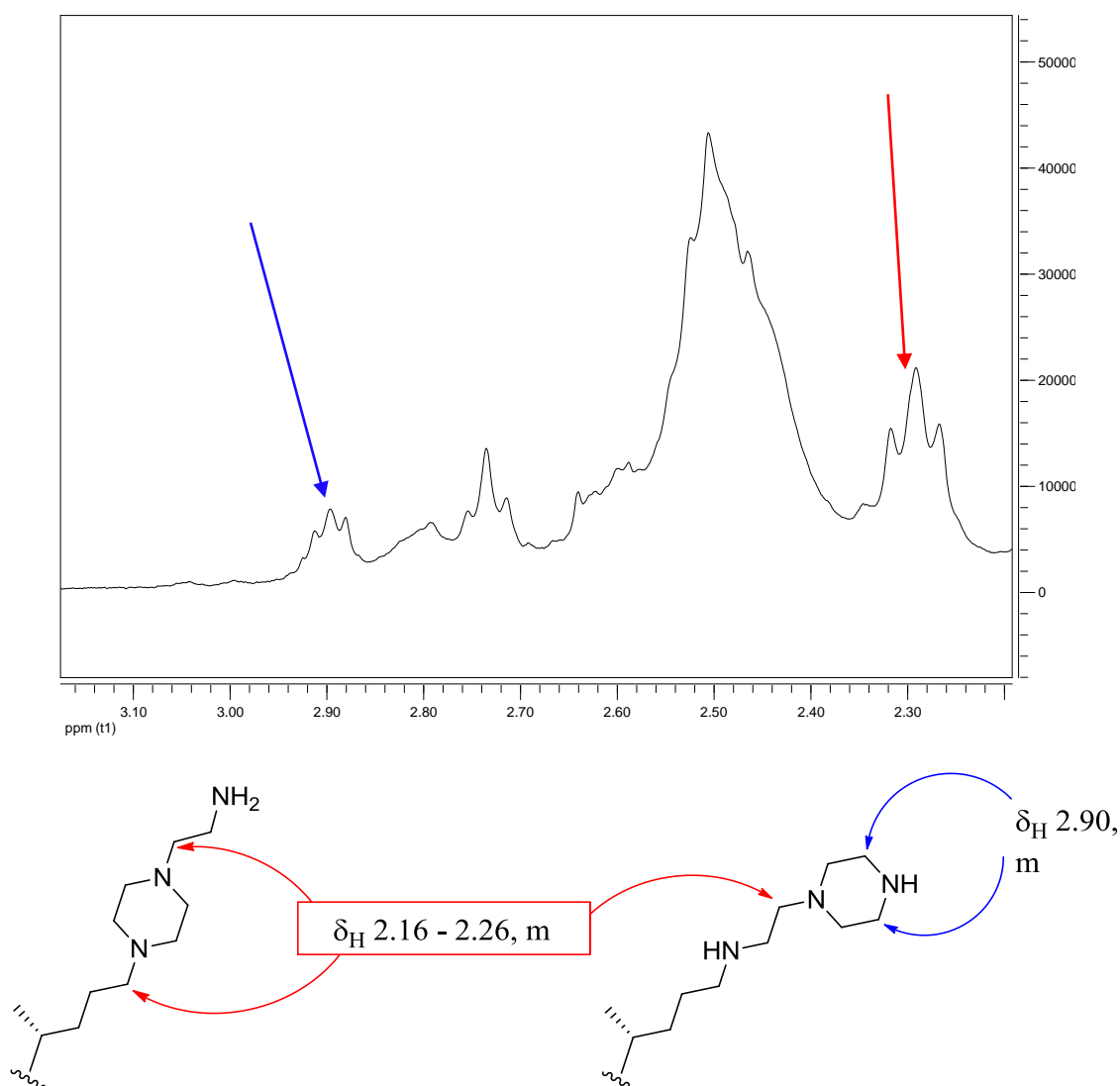
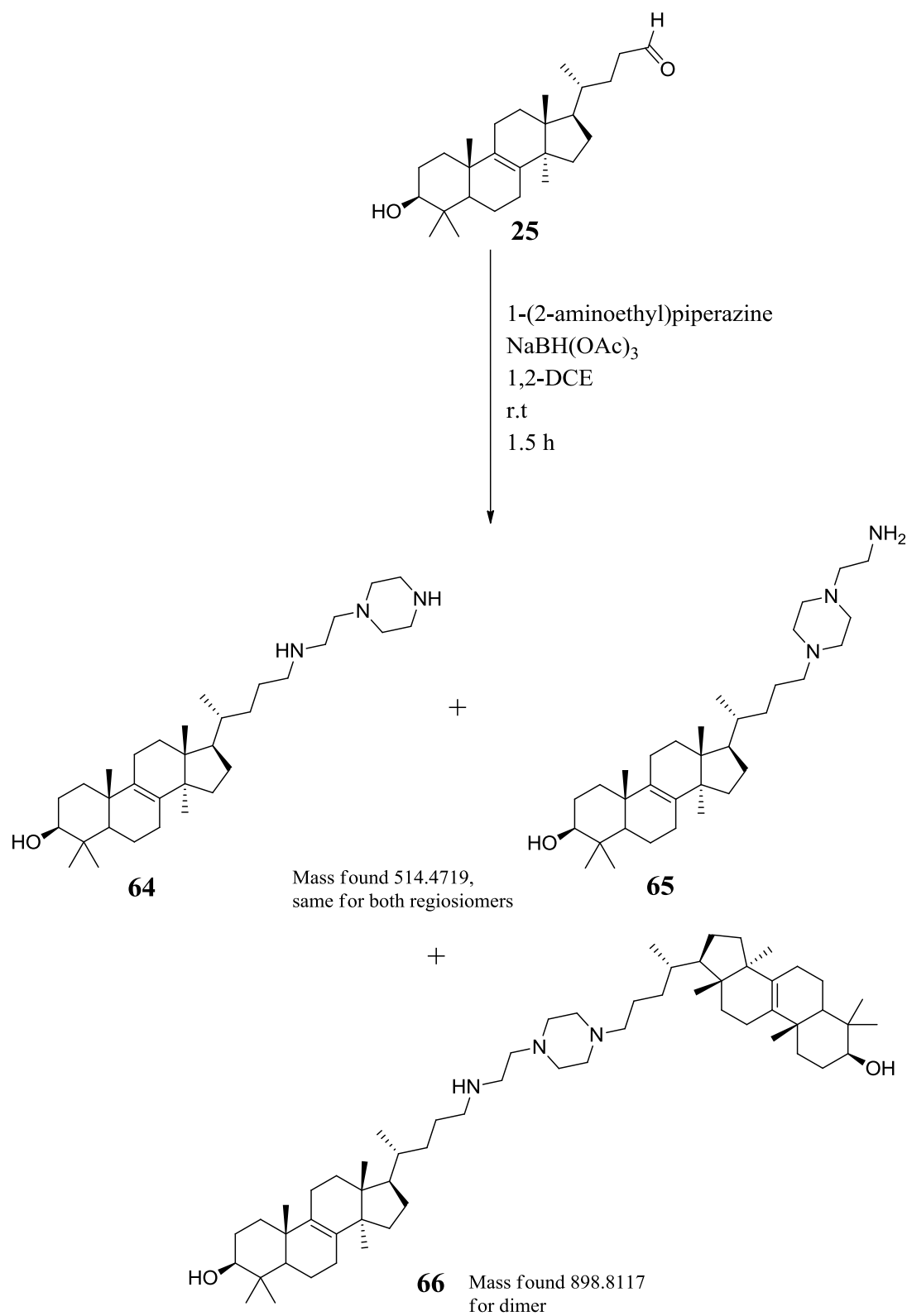


Figure 2.53: ^1H NMR spectrum of the mixture of **61**, **62** and **63**

A quantity of the crude material was separated from the bulk and allocated for testing. The remainder was used to attempt purification, however all attempts to separate these regioisomers by recrystallisation and column chromatography were unsuccessful, leading to the eventual loss of the product.

The reductive amination using 1-(2-aminoethyl)piperazine was repeated with starting aldehyde **25**. In this case a similar result emerged, and a mixture of regioisomers **64** and **65**, and dimer **66**, was formed, as an off white solid (Scheme 2.40).



Inseparable mixture

Scheme 2.40

The formation of dimer **66** is confirmed by high resolution mass spectrometry, as the mass found of 898.8117 corresponds to the exact mass of the unsymmetrical dimer. The formation of regioisomers **64** and **65** is seen by a peak at 514.4719 in the high resolution mass spectrum. As discussed in detail above, the signals shown in Figure 2.54 are indicative of the formation of both regioisomers.

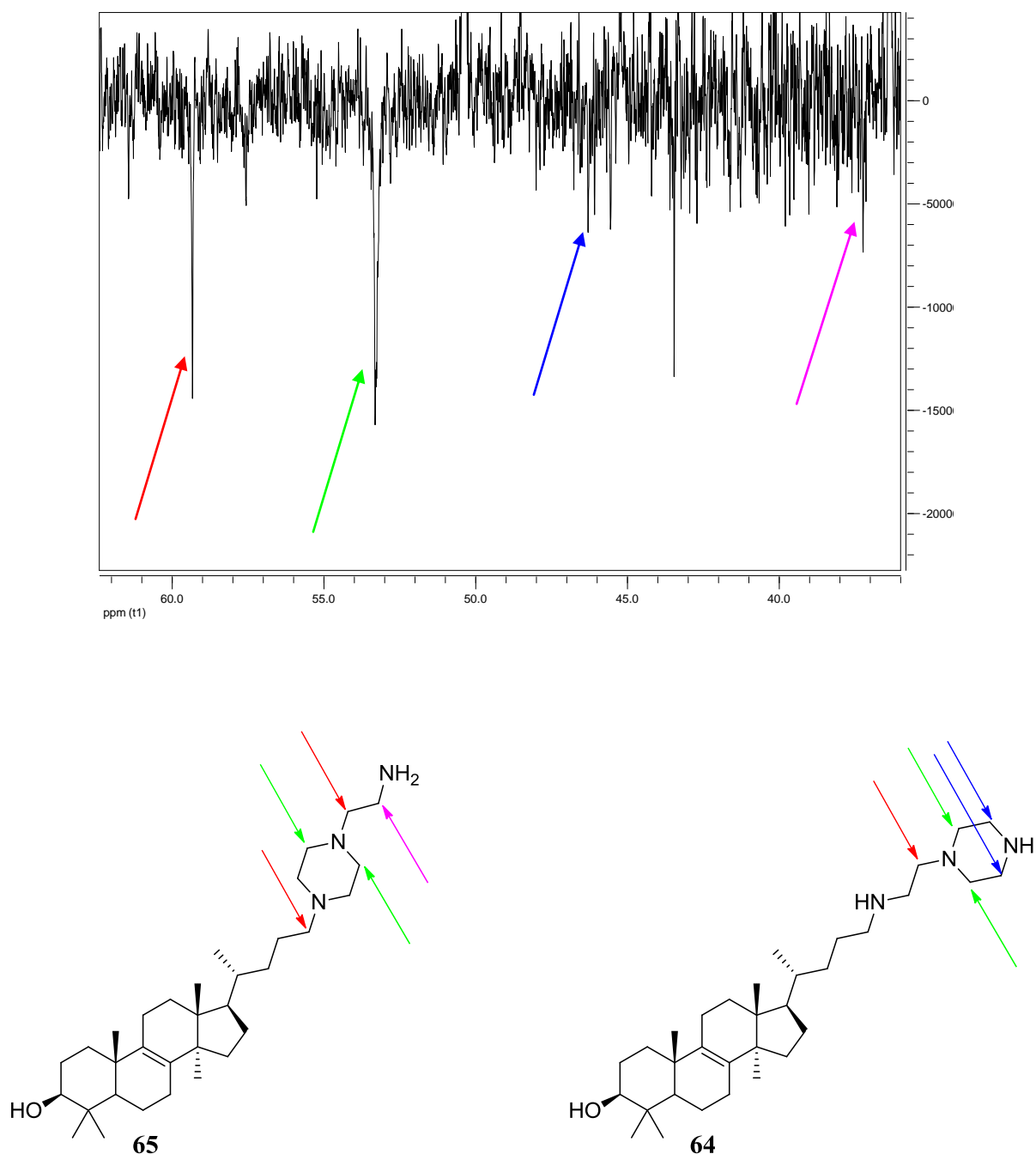


Figure 2.54: ^{13}C NMR spectrum of **65** and **64** run in the DEPT 135 mode

Note: Structure of dimer **66** is removed from Figure 2.54 for clarity. Signals follow the same pattern as dimer **62** (Figure 2.52)

A similar pattern occurs in the ^1H NMR spectrum (Figure 2.55), where the signal at ~ 2.90 ppm is the only distinctive signal in the mixture of compounds, being assigned to the two CH_2 groups adjacent to the free NH of the piperazine ring in regioisomer **64**.

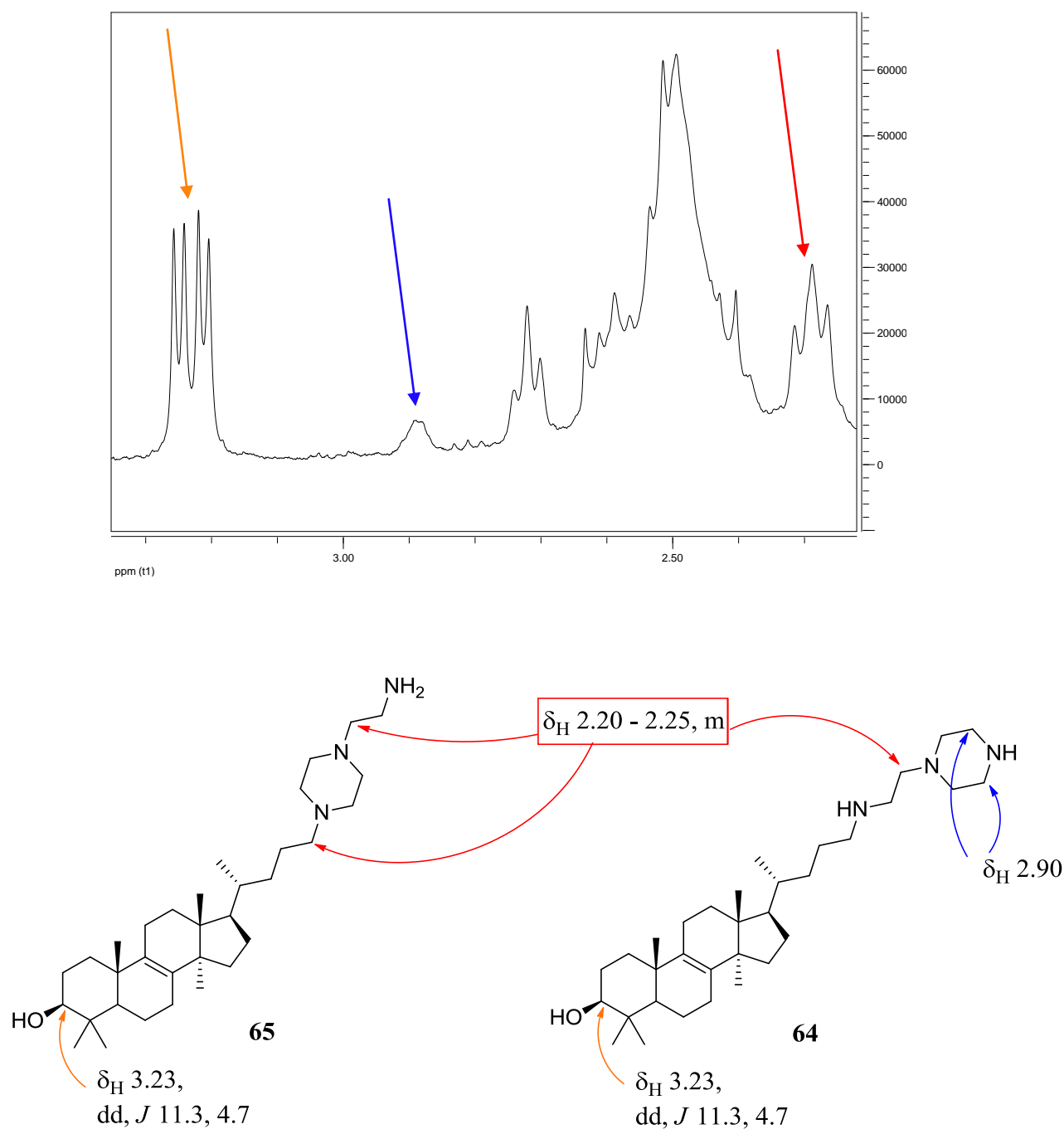
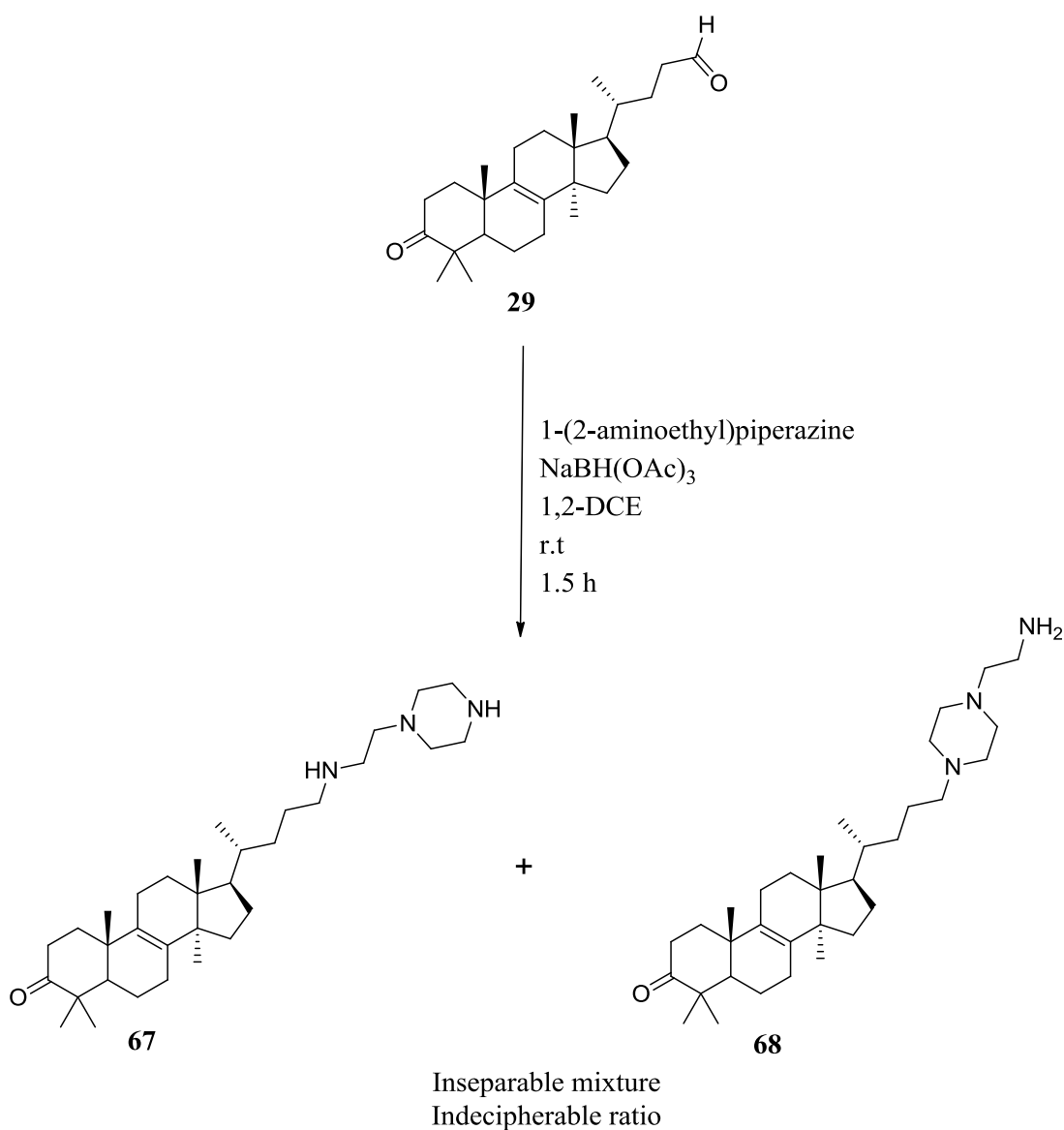


Figure 2.55: ^1H NMR spectrum of **65** and **64**

Separation of this mixture of regioisomers was not attempted in this case, given the loss of all product in the attempted purification of the C-3 acetate analogues **61**, **62** and **63** by recrystallisation and column chromatography.

In correlation with our 1-(2-hydroxyethyl)piperazine series, we extended the reductive aminations in this series to include the C-3 ketone derivative, yielding regioisomers **67** and **68** (Scheme 2.41). This work was carried out at approximately the same time period as previous reductive aminations involving 1-(2-aminoethyl)piperazine, where the regioisomer formation and dimerisation issue had not yet come to light.



Scheme 2.41

No evidence for the presence of the dimer is seen in the high resolution mass spectrum in this case, however its formation and subsequent fragmentation upon electrospray ionisation cannot be ruled out. Turning to the ^1H NMR spectrum (Figure 2.56), the broad singlet at 2.95 ppm which correlates with the CH_2 groups adjacent to the free NH group on the piperazine ring, is of a higher intensity relative to the other two signals at 2.82 ppm and 2.32 ppm. This leads us to believe that **67** is formed in a much higher proportion in this instance. The ratio of regioisomers remains indecipherable as we cannot rule out the formation of the dimer in this instance, as discussed.

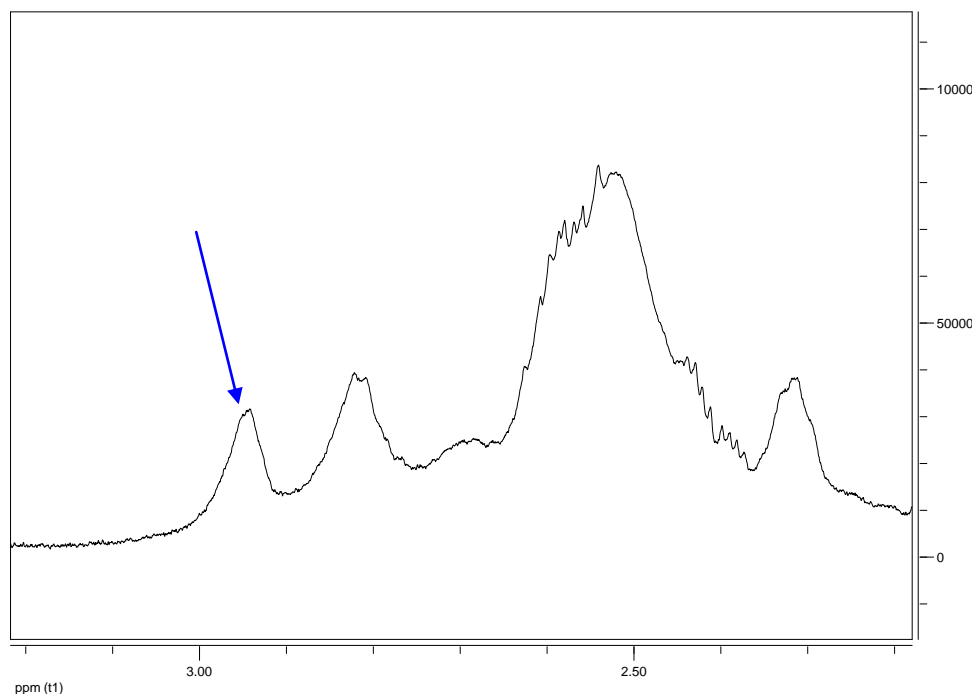
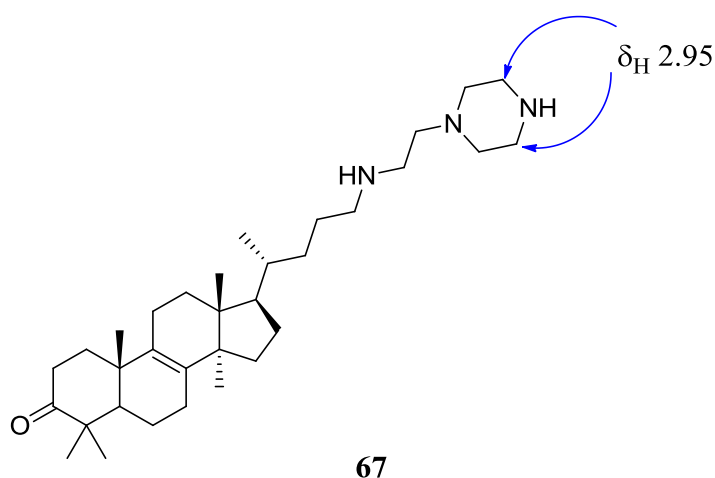
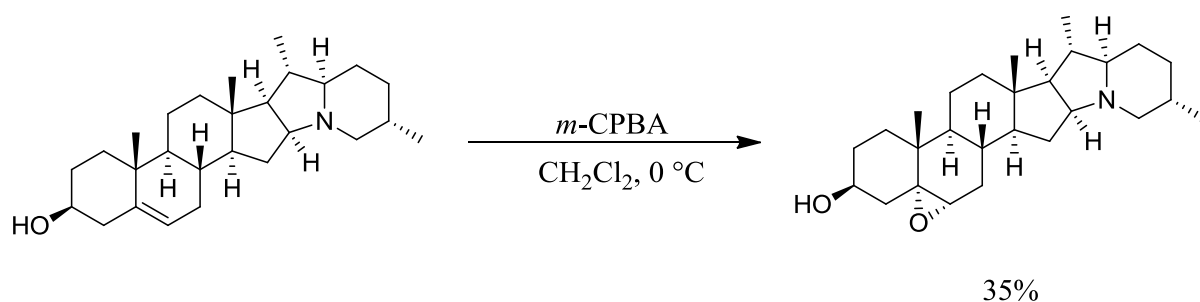


Figure 2.56: ^1H NMR spectrum of **67** and **68**

In summary, and with the benefit of hindsight, we are aware that 1-(2-aminoethyl) piperazine was a problematic choice of amine starting material, as it led to the formation of regioisomers and dimerisation. Upon reflection of this body of work, we conclude that the inseparability of these complex mixtures of products renders futile reductive amination with starting materials containing diamine functionalities due to the primary and secondary amine sites. It may be possible in future work to rationalise this regiospecificity through modifications of reaction conditions. In our case, the products were retained for biological evaluation.

2.4.5 *N*-oxide Study

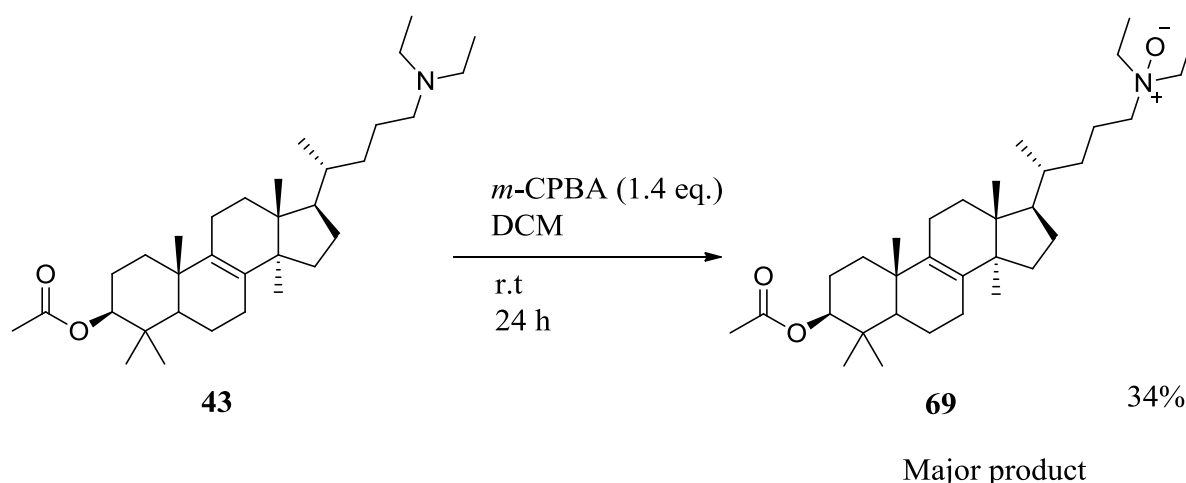
Having successfully synthesised amine derivatives of lanosterol that showed promising biological activity, we were enthusiastic to ascertain their chemical reactivity towards oxidation. The opportunity to synthesise novel compounds by oxidising our amine derivatives was an exciting prospect, as it would combine the most important aspects of our research to date. Previous work in our group, carried out by Dr. Sinead Milner, had been focused on the oxidation of solanidine in the presence of tertiary nitrogens, the results from which would be of great benefit to us in this study. Dr. Milner found that the major reaction pathway under these conditions was the oxidation of the tertiary nitrogen in preference to the alkene, although, upon purification of the crude material, a fraction was isolated which contained only a product of C-5,6 epoxidation (Scheme 2.42).



Scheme 2.42

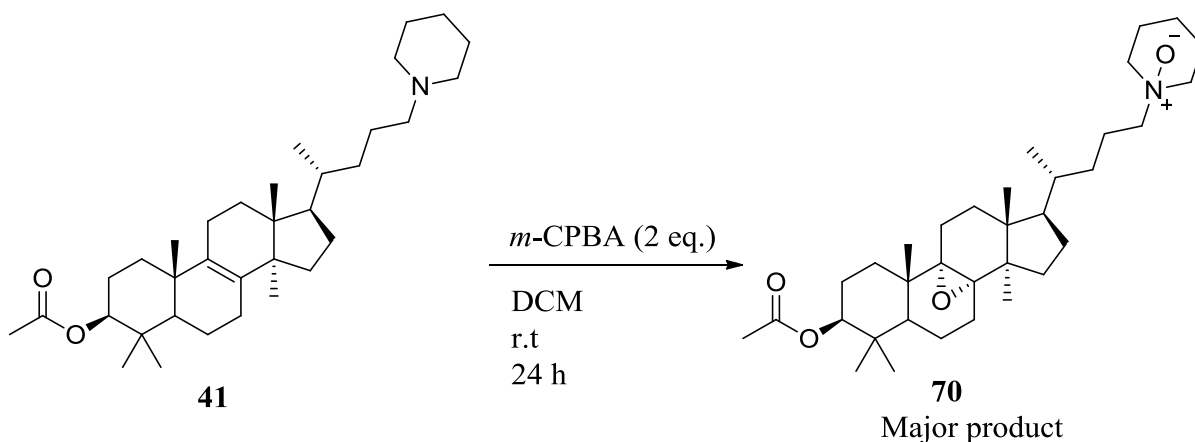
Given the results of our biological screen on the amine derivatives, we wanted to incorporate the diethylamine and piperidine functionalities in as much as possible in further derivatives synthesised, as these had given the most promising results. An initial aim was to synthesise the C-8,9 epoxide of these derivatives given that an epoxide was the most active functionality from the first screen. However, we were unsure about the order of oxidation of this compound, given that the alkene is significantly more sterically hindered than that in solanidine, and the amine is more exposed.

The first test reaction carried out was the oxidation of diethylamine derivative **43** with 1.4 equivalents of *m*-CPBA (Scheme 2.43). Upon reaction completion and work up, the crude material was observed to be a dark orange solidifying oil. Recrystallisation from ethyl acetate yielded the product **69** as a light orange solid in 34% yield. ^1H NMR spectroscopic analysis showed a downfield shift in 24-CH₂ and both diethylamine CH₂ groups, indicating the oxidation of the adjacent nitrogen to an *N*-oxide. 24-CH₂ shifted downfield from 2.32 – 2.51 ppm to 3.20 – 3.40 ppm. A similar pattern was seen for the diethylamine CH₂ groups, shifting from 2.52 – 2.67 ppm to 3.42 – 3.57 ppm. From these shifts in methylene groups adjacent to the nitrogen, it can be reasonably concluded that the *N*-oxide **69** had formed. However, the ^{13}C NMR spectrum would be the most elucidatory when determining that the alkene at C-8,9 remained unreacted. Peaks at 134.3 and 134.4 ppm showed that epoxidation had not taken place across this double bond and the alkene was still present.



Scheme 2.43

The piperidine derivative **41** was also oxidised with *m*-CPBA, however, in this instance, two equivalents of oxidant were used, in order to allow both oxidisable sites to react and form **70** (Scheme 2.44). Difficulty was encountered in the work up of this reaction, as several emulsions formed in the sodium bicarbonate washing process. When the crude material was retrieved from the emulsion and concentrated under reduced pressure it was found to also be an orange oil. Unfortunately, multiple crystallisations from hexane and ethyl acetate did not remove the trace amounts of *m*-CPBA in this case. Therefore, an accurate yield could not be calculated, as it would not be a true reflection of the reaction yield. The ^1H NMR spectrum showed similar downfield shifts of the methylene groups adjacent to the nitrogen, confirming that the *N*-oxide had formed. Unfortunately, ^{13}C NMR analysis was not possible due to substantial loss of product following multiple recrystallisations, therefore high resolution mass spectrometry was used to confirm the presence of the epoxide at the C-8,9 position. The calculated mass of 544.4366 for **70** correlated very closely with the found mass of 544.4367. As we had anticipated, the increase in *m*-CPBA led to oxidation at both available sites.

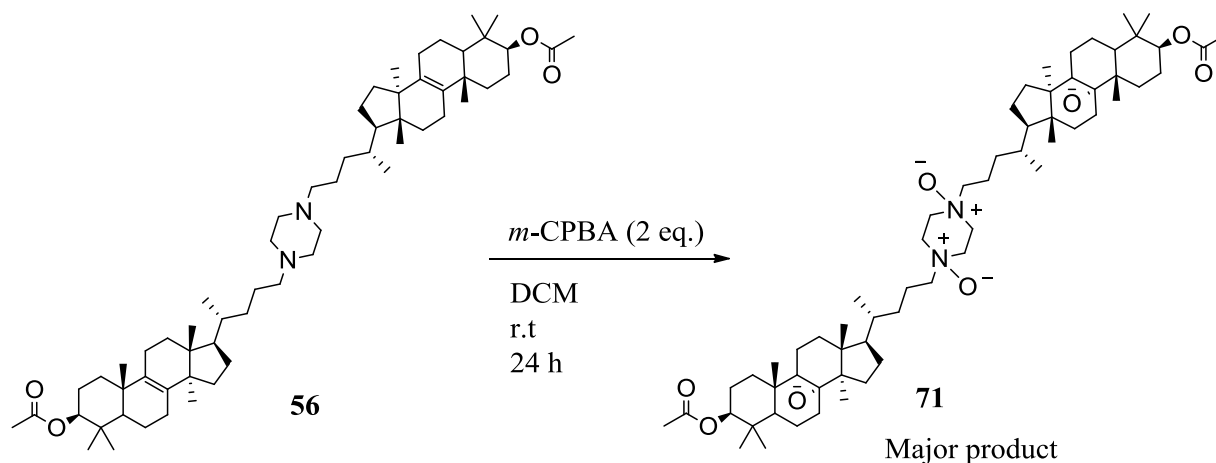


Scheme 2.44

These two test reactions had given us a valuable insight into this chemistry. As shown above, the tertiary nitrogen site is the first to be oxidised with the lesser equivalency of *m*-CPBA. It required two equivalents of the oxidant to react with both the tertiary nitrogen and the alkene, and these compounds were found to be very difficult to purify due to the excess *m*-CPBA.

Two further test reactions were carried out on piperazine and morpholine derivatives to conclude this chemistry.

Firstly, piperazine dimer derivative **56** was reacted with two equivalents of *m*-CPBA (Scheme 2.45) to yield **71**. This reaction was carried out to verify that the epoxide had formed at the C-8,9 position by ^{13}C NMR analysis, as insufficient quantities of **70** had prevented us obtaining this information. Again, purification was difficult, and recrystallisation of the product was unsuccessful. However, as our interest lay in the proof of the formation of the epoxide, we were keen to analyse the ^{13}C NMR spectrum, and the observation of two epoxide peaks at 67.8 and 70.4 ppm was a positive result. The presence of one alkene signal at 134.3 ppm shows that there were trace quantities of the alkene remaining.

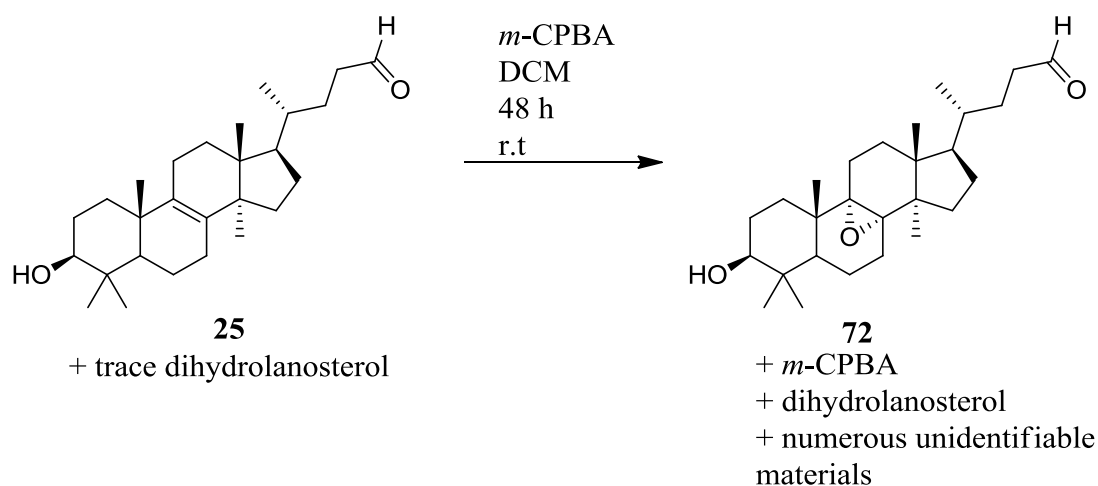


Scheme 2.45

The final test reaction carried out in this section was to explore the possibility of the formation of these compounds *via* an alternative route. Thus far, the reductive aminations had preceded epoxidation and *N*-oxidation. It was therefore investigated if the C-8,9 epoxide could be formed first, with subsequent reductive amination taking place at the C-24 aldehyde.

Aldehyde **25** was reacted under the standard conditions of *m*-CPBA in dichloromethane in order to epoxidise the internal C-8,9 alkene bond (Scheme 2.46). The reaction was stirred

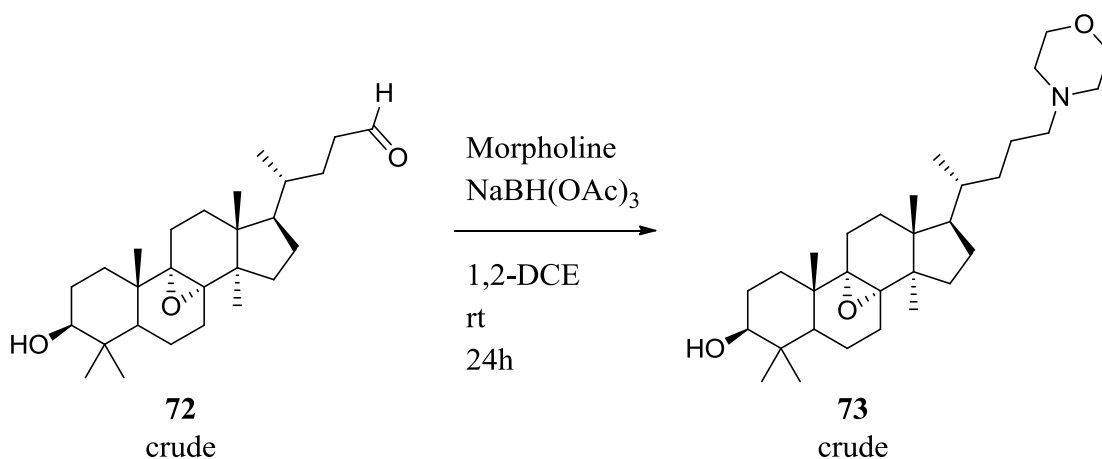
over a 48 hour period. After this time the work up was found to be difficult, and several emulsions were formed. Extensive brine washes eventually broke up the emulsion and the desired product **72** was isolated in crude form. However, the product contained residual *m*-CPBA which could not be removed after multiple sodium bicarbonate washes and several recrystallisation attempts. Characteristic signals for the desired product in the ^1H NMR spectrum are the 2H multiplet between 2.35 ppm and 2.55 ppm assigned to 23-CH₂, the 1H doublet of doublets at 3.21 ppm assigned to 3-CH and the triplet at 9.77 ppm which is the aldehyde proton. This aldehyde triplet integrates for ~0.3 when compared to the 1H integral of the doublet of doublets at 3.21 ppm, indicating that the desired aldehyde accounts for ~30% of the product mixture. Characteristic epoxide signals at 68.1 ppm and 70.7 ppm verify that the C-8,9 alkene did undergo epoxidation.



Scheme 2.46

When this epoxy-aldehyde **72** was synthesised, albeit in crude form, the reductive amination using morpholine was attempted (Scheme 2.47). The reaction time was increased to 24 h, to account for the lower quantity of aldehyde present in the starting material. Upon reaction work up, the ^1H NMR spectrum of the crude material showed impurities, including *m*-CPBA, dihydrolanosterol and numerous unidentifiable products, were present in addition to the desired product **73**. Purification was not attempted, as limited quantities of material remained, and it was our priority to gather as much raw analysis as possible with this material. Encouragingly, the ^1H NMR spectrum showed that the reaction had gone to completion, as no aldehyde remained in the product. The characteristic methylene groups adjacent to the oxygen in morpholine were present, as seen by 4H broad singlet at 3.82 ppm. Further evidence for the

formation of the product was found in the ^{13}C NMR spectrum, where the morpholine N-CH_2 and O-CH_2 groups were seen in the expected regions at 52.6 ppm and 65.6 ppm. The quaternary carbon signals at 68.1 ppm and 70.9 ppm were evidence for the epoxide at this position remaining present after the reductive amination reaction. There was no evidence to suggest that the amine nucleophile had opened the hindered C-8,9 epoxide. Rather, the nucleophile undergoes reductive amination with the less sterically hindered aldehyde.



Scheme 2.47

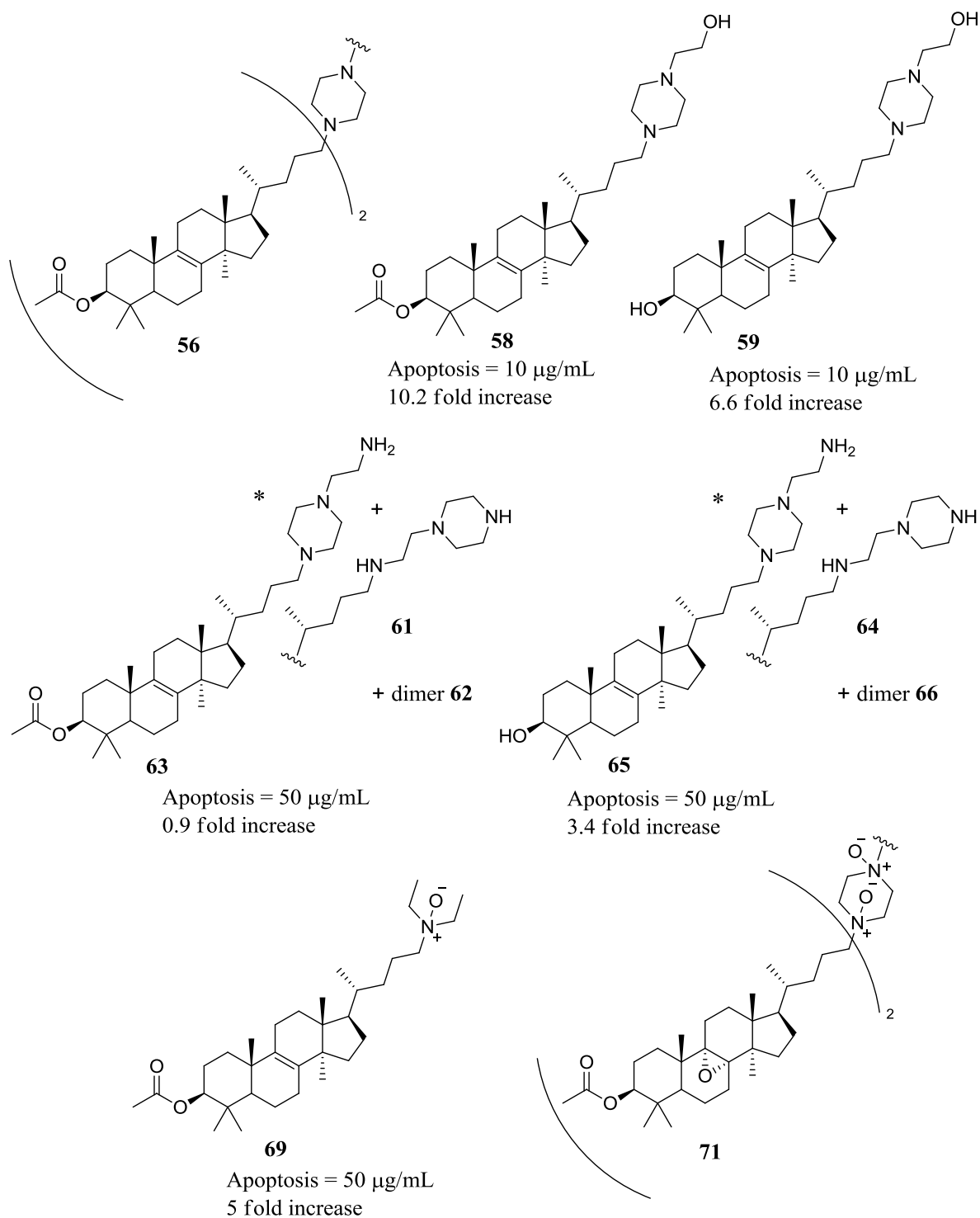
This reaction has shown that the prior epoxidation of the C-8,9 alkene, followed by reductive amination could become a viable pathway in the synthesis of novel amines with higher oxygenation. Unfortunately this morpholine derivative **73** was insufficiently pure for biological evaluation or further derivitisation, for example the synthesis of its corresponding *N*-oxide.

This series of reactions had been of limited success in terms of producing products with the high level of purity required for biological testing. However, the range of reactions carried out give us an insight into this chemistry for future work, and two main conclusions have been drawn from this *N*-oxide study.

Firstly, the nitrogen of the tertiary amine is the first point of oxidation when a compound containing alkene and amine sites is treated with *m*-CBPA. Additionally, preliminary work has shown that it is possible to perform the reductive amination reaction without unwanted reactions at the C-8,9 epoxide. Optimisation of this work would open up a wide range of synthetic scope for novel amine derivatives.

2.4.6 Biological testing 4: Amine Derivatives of Lanosterol

Table 2.14



* Results are presented for a mixture of **61**, **62** and **63**, and a mixture of **64**, **65** and **66**.

% Viability of U937 cells					
	200 $\mu\text{g/mL}$	100 $\mu\text{g/mL}$	50 $\mu\text{g/mL}$	10 $\mu\text{g/mL}$	5 $\mu\text{g/mL}$
56				94.41 ± 1.29	96.96 ± 2.85
58				14.10 ± 0.85	53.42 ± 3.46
59				5.89 ± 0.48	5.83 ± 0.37
61, 62, 63			6.51 ± 0.49	46.91 ± 4.20	78.76 ± 4.37
64, 65, 66			7.35 ± 0.71	91.43 ± 4.10	93.48 ± 2.86
69			5.78 ± 0.70	56.99 ± 4.37	75.12 ± 3.98
71	48.89 ± 5.63	51.90 ± 0.43	52.10 ± 0.71	86.26 ± 1.60	

Percentage Viability of U937 cells at
Compound Concentration of 10 $\mu\text{g/mL}$

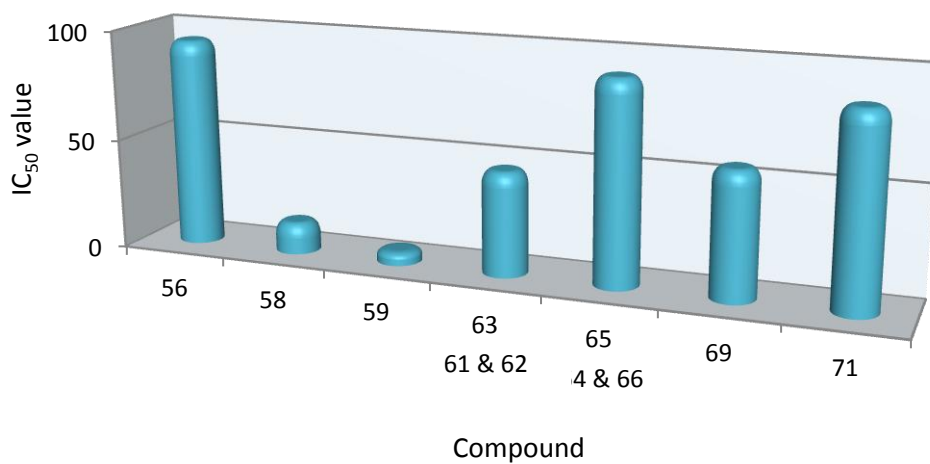
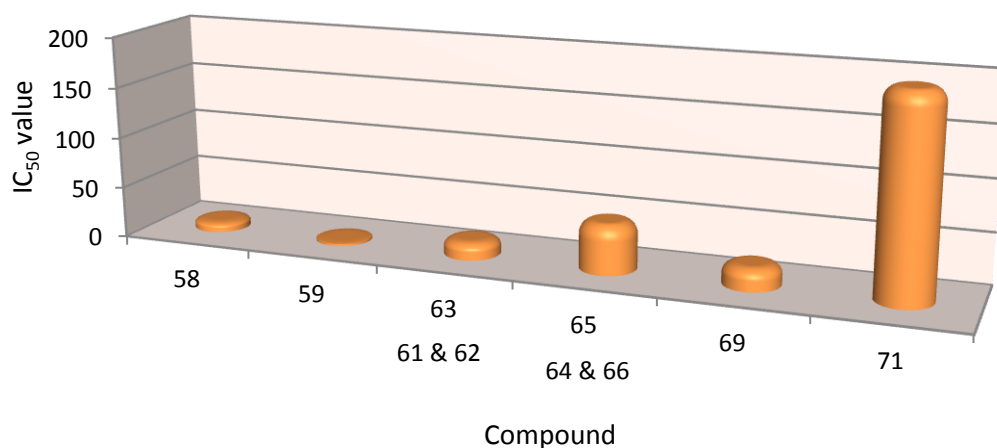


Figure 2.57: Percentage viability of U937 cells at compound concentration of 10 $\mu\text{g/mL}$ for compounds **56**, **58**, **59**, **61-63**, **64-66**, **69** and **71**.

	IC ₅₀ values	
	μg/mL	μM
56	-	-
58	5.2	9.3
59	1.4	2.7
61, 62, 63	9.2	16.6
64, 65, 66	24.5	47.7
69	10.9	21.1
71	105.8	194

Table 2.15

IC₅₀ values in U937 cells μMFigure 2.58: IC₅₀ values of compounds **56**, **58**, **59**, **61-63**, **64-66**, **69** and **71** (μM).

The standout biological result from this screen is undoubtedly the activity of **59**. At a concentration of just 5 μg/mL, the percentage viability of U937 cells is ~5%. This is an improvement on all other compounds in our previous screens. The IC₅₀ result for this compound is also excellent, **59** having an IC₅₀ value of 2.7 μM. When this IC₅₀ value is compared to Etoposide, a topoisomerase II inhibitor on the market, which has an IC₅₀ value of 1.5 μM in these U937 cells, its significance is apparent.

We were particularly delighted with the biological results of this compound because it had been strategically designed from knowledge gained in the previous screens. As discussed earlier, we had selected an amine with a free alcohol group due to the fact that the alcohol functionality had given promising results in compounds **42** and **44**. This excellent result, based on the strategic manipulation of our compounds, is a great triumph of our chemical and biological collaboration, and indeed the project as a whole.

The second most active compound of our biological screen was the analogous acetate derivative **58**, having a percentage viability of ~ 14% at 10 $\mu\text{g/mL}$ and an IC_{50} value of 5.2 $\mu\text{g/mL}$. This is an interesting result to compare with previous screens. For instance, other C-3 acetate derivatives proved much less biologically active than their analogous C-3 alcohols. However, in this case, although **58** contains the C-3 acetate group, the alcohol in the side chain is improving its biological activity.

Compounds which showed extremely poor biological activity were the dimers **56** and **71**, showing negligible IC_{50} values, and poor percentage viability results. This is to be expected due to the large steric bulk of the dimer and consequent poor solubility and hydrogen bond donating properties.

Compounds **61** - **66** were tested as mixtures of the major and minor regioisomers, and the dimer in both cases, as detailed in the footnote to Table 2.14. While these compounds show poorer biological activity than their comparable hydroxyethyl analogues, it is interesting to compare them in terms of acetate and alcohol derivatives. A striking result is that, in this case, the acetate derivative shows better activity than the corresponding alcohol in terms of IC_{50} values, displaying IC_{50} 's of 9.2 $\mu\text{g/mL}$ and 24.5 $\mu\text{g/mL}$ respectively. This result is in contrast to our other compounds tested, where the C-3 alcohol was the most active of comparable analogues. Although this is an unexpected result, these compounds were not tested in pure form. Therefore, further studies must be carried out into the selective formation of the desired regioisomer in order to confirm these trends in biological activity.

2.4.7 Sugar chemistry

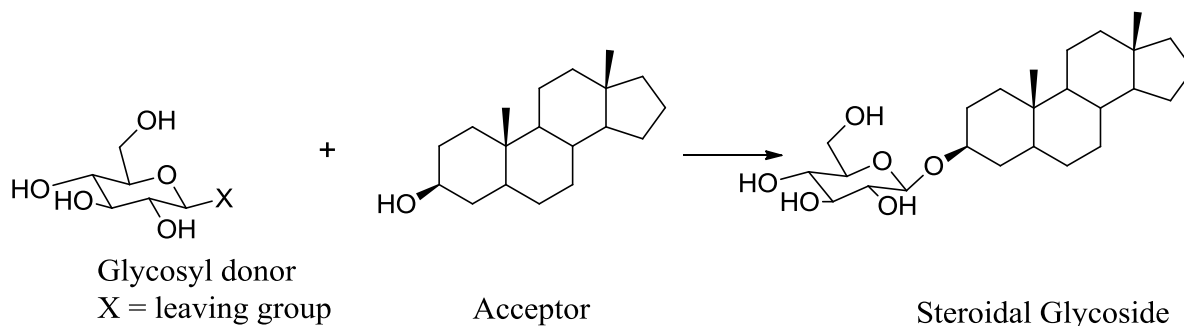
2.4.7.1 Glycosylation of 3 β -hydroxy-5 α -4,4,14-trimethyl-24-piperadino-chol-8-ene 42

The glycosylation of steroids has been reported in a review by Pellissier in 2004.⁴² Prior to this report, more general *O*-glycosylation methods, which were applied to the synthesis of natural products, have been documented in several publications.⁴³⁻⁴⁶

It is well known that an important class of drugs are glycosides, and the presence of the sugar moiety greatly enhances the biological properties of these compounds. The sugars play a key role in the interaction of drugs with their receptors, as well as significantly affecting the pharmacokinetics of the drug in question.⁴⁷ Moreover, glucuronides are often the final form of a drug or xenobiotic eliminated from the body, demonstrating an important detoxification role.⁴²

Steroidal glycosides constitute a diverse class of compounds which have been isolated from a wide variety of plant and animal species.^{48,49} They display a wide range of pharmacological effects such as antiviral,^{50,51} antibacterial⁵² and anti-inflammatory activity.⁴² While extensive research has been conducted into the synthesis of oligosaccharides and steroids, the challenge in the synthesis of steroidal glycosides lies in the construction of the glycosidic linkage at the anomeric carbon.

Despite a wealth of knowledge of carbohydrate chemistry,⁵³⁻⁵⁶ the stereoselective formation of *O*-glycosidic bonds between carbohydrates and steroids is relatively cumbersome. This is due to the low reactivity of the secondary alcohol in the steroid moiety, and the necessity to activate the glycosyl donors.⁴² The scheme for a general glycosylation reaction is shown below, (Scheme 2.48)

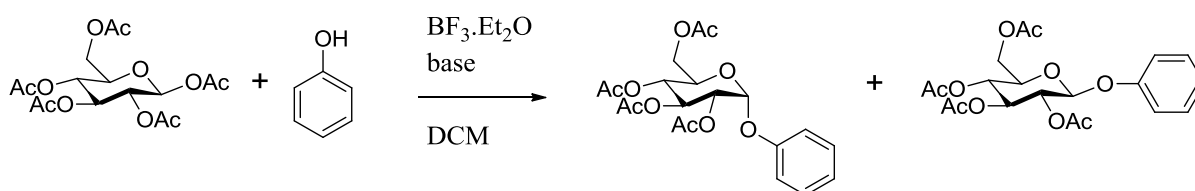


Scheme 2.48

Our interest in glycosylation of steroids stems from the literature precedent that enhancement of the biological properties in naturally occurring structures and their mimetics.⁴⁷ As we had accomplished our main objectives in this section, i.e. the synthesis of amine derivatives of lanosterol, their biological evaluation, and the determination of their chemical reactivity, we were keen to investigate the glycosylation of our steroid derivatives.

Lee *et al.* describe the *O*-glycosylation of phenol and phenol derivatives using penta-*O*-acetyl- β -D-glucopyranose in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ and an organic base resulting in both high yield and high β -stereoselectivity.⁵⁷

Table 2.16

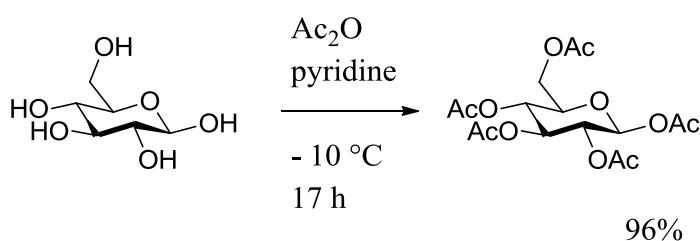


Entry	$\text{BF}_3 \cdot \text{Et}_2\text{O}$ (eq)	Base (eq)	Reaction time (h)	Yield (%)	Ratio (α/β)
1	2.5	None	5.5	72	18/82
2	2.5	Et_3N	3	89	7/93
3	2.5	Et_3N	3	92	2/98
4	2.5	TMG ^a	5	89	4/96
5	2.5	2,6-lutidine (0.5)	5.5	91	3/97
6	2.5	DTBMP ^b (0.5)	7	88	3/97
7	2.5	Et_3N	5	87	3/97
8	1.5	Et_3N	11	72	1/99
9	1.5	Et_3N	5.5	87	2/98
10	4	Et_3N	2	80	2/98
11	10	Et_3N	0.5	92	9/91

a: 1,1,3,3-tetramethylguanidine. b: 2,6-di-*tert*-butyl-4-methylpyridine

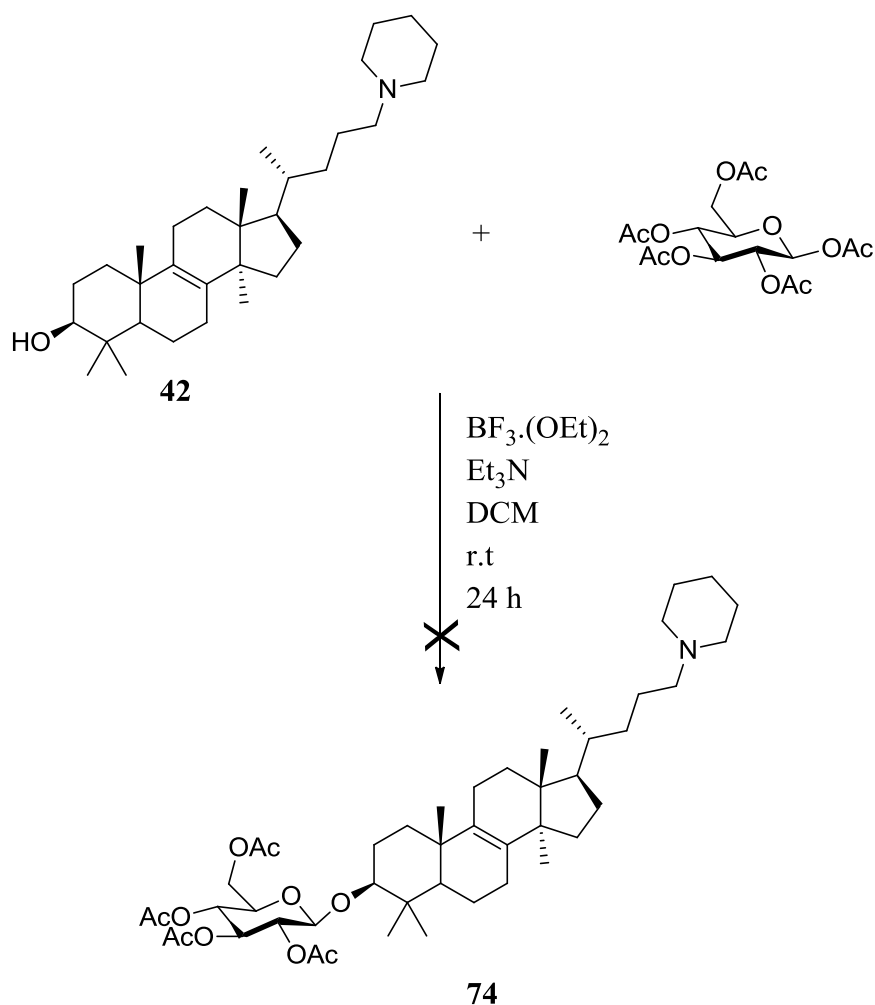
As can be seen from Table 2.16, Entry **3**, glycosylation of phenol in the presence of 2.5 equivalents of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ and 0.5 equivalent of triethylamine to penta-*O*-acetyl- β -D-glucopyranose gave the highest yield and excellent β -stereoselectivity. Thus we employed these initial conditions in our attempted glycosylation of a steroid.

Penta-*O*-acetyl- β -D-glucopyranose was kindly supplied to our research group by Lorna Lennon, under the supervision of Dr. Humphrey Moynihan, Chemistry Department, UCC. The sugar was synthesised in 96% yield as shown in Scheme 2.49.^{58,59}



Scheme 2.49

Compound **42** was the natural choice for method development in this instance, as it had shown the most superior biological activity of our compounds screened in this series in terms of IC_{50} value. Additionally, the side chain tertiary amine functionality would be comparable to solanidine and the C-3 alcohol group would be susceptible to glycosylation. Thus, we attempted *O*-glycosylation of **42** with penta-*O*-acetyl- β -D-glucopyranose using standard Lewis acid coupling conditions outlined by Lee *et al.* in an attempt to synthesise **74**.⁵⁷ Boron trifluoride diethyletherate and triethylamine were employed in the glycosylation reaction (Scheme 2.50)

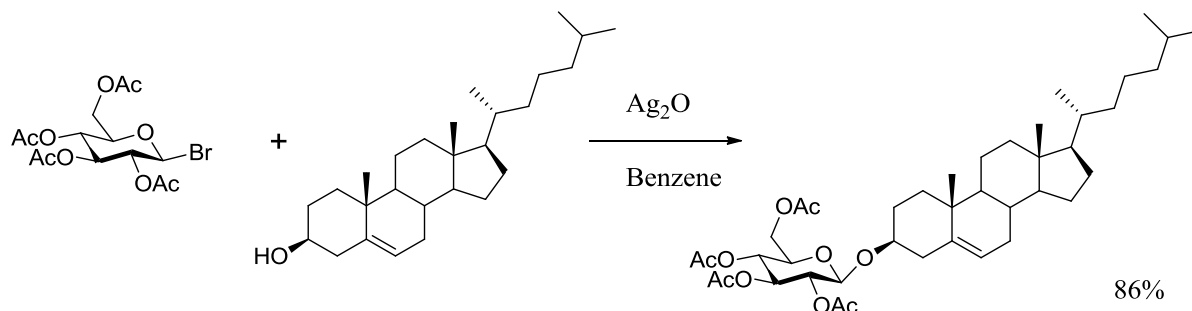


Scheme 2.50

Upon reaction completion and work up, inspection of the ^1H NMR spectrum revealed no traces of desired product had been formed. This is evident by the ^1H doublet at 5.72 ppm, corresponding to 1-CH of starting material penta-*O*-acetyl- β -D-glucopyranose, appearing at an identical chemical shift in the ^1H NMR spectrum. Furthermore, the 3-CH doublet of doublets appearing at 3.23 ppm in the ^1H NMR spectrum of starting amine **42** would also have been expected to shift, which was not observed. The ^{13}C NMR spectrum also shows evidence that the reaction was unsuccessful, most strikingly in the fact that five carbonyl peaks remain in the product, corresponding to the five acetate groups. One of these acetate groups would have been expected to disappear had the reaction been successful.

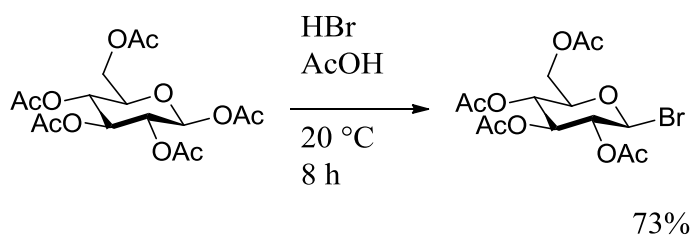
Despite our disappointment with this initial attempt at glycosylation, we were keen to persist with this chemistry. The previously mentioned review⁴² detailed a procedure for the

formation of steroidal glycosides using silver oxide in the Koenigs-Knorr reaction. This method was successful in the glycosylation of cholesterol, first reported by Schneider *et al.* in 1969 (Scheme 2.51).⁶⁰



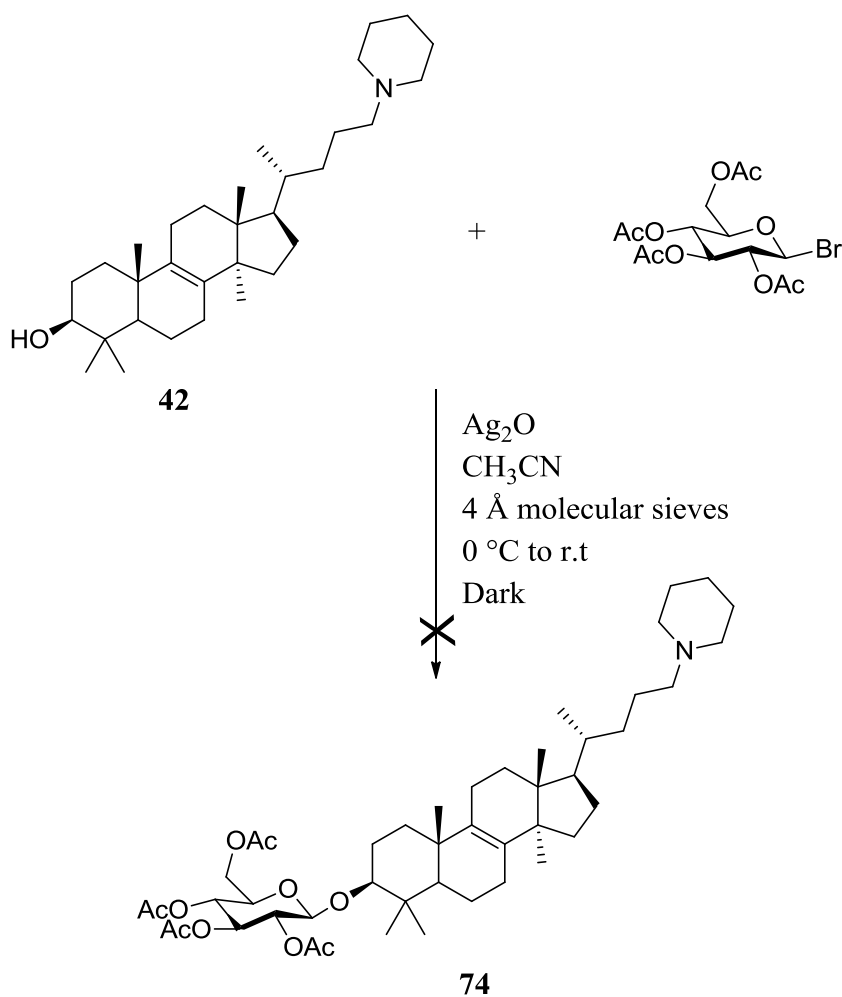
Scheme 2.51

Again, the required sugar 1-bromo-1-deoxy-2,3,4,6-tetra-*O*-acetyl-β-D-glucose was kindly supplied to our research group by Lorna Lennon, under the supervision of Dr. Humphrey Moynihan, Chemistry Department, UCC. The sugar was synthesised in 73% yield as shown in Scheme 2.52.⁶¹



Scheme 2.52

Thus, we reacted 1-bromo-1-deoxy-2,3,4,6-tetra-*O*-acetyl-β-D-glucose with **42** using silver oxide (Scheme 2.53).



Scheme 2.53

Unfortunately, upon reaction completion and work up, ^1H NMR analysis revealed that the crude reaction material consisted of a mixture of starting sugar and steroid, with no evidence of formation of the desired compound **74**. The lack of any visible shift of either the 1-CH signal of 1-bromo-1-deoxy-2,3,4,6-tetra-*O*-acetyl- β -D-glucose at 6.62 ppm or the 3-CH signal of **42** at 3.24 ppm is evidence that the coupling had been unsuccessful.

We did not pursue this area of the project further, however, the glycosylation methodology will be investigated in future work, as the synthesis and biological evaluation of these compounds is a potentially important aspect of the research going forward.

2.5 Stigmasterol Chemistry

2.5.1 Stigmasterol

Stigmasterol **9** belongs to a class of steroidal compounds known as phytosterols, an umbrella term for plant stanols and sterols. The importance of phytosterols lies in their ability to lower cholesterol, and thus phytosterol enriched foods and dietary supplements have been popular in recent decades. As discussed in the project outline, stigmasterol is naturally present in the human diet, consisting of ~ 3% of phytosterol intake,⁶² and we were attracted to working with this compound as we were keen to establish a food related aspect to our research given that our biological collaborators have a strong interest in food chemistry and have several publications in this area. As lanosterol is found naturally in wool wax, and lanostane derivatives used in cosmetics, we were unable to establish a food angle to this research. Additionally, stigmasterol had a chemical structure suitable for modification by our established synthetic routes, namely cleavage of the side chain alkene to form an aldehyde and subsequent reductive amination, which could lead to an analogous series of tertiary amines similar to those derived thus far for lanosterol.

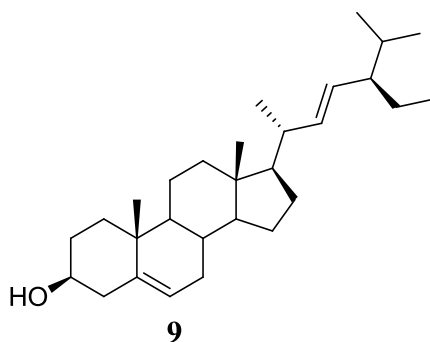


Figure 2.59 Stigmasterol **9**

Recent literature reports have detailed the cytotoxic effects of oxygenated derivatives of stigmasterol in U937 cells.^{34,63} These derivatives were synthesised by exploiting the alkene functionalities of stigmasterol to form epoxides, diols, and allylic oxidation products. The most biologically active of these derivatives in the U937 cells was, the previously discussed, diepoxystigmastane **39**, which gave a ~ 51% viability of cells at a concentration of 30 μ M.

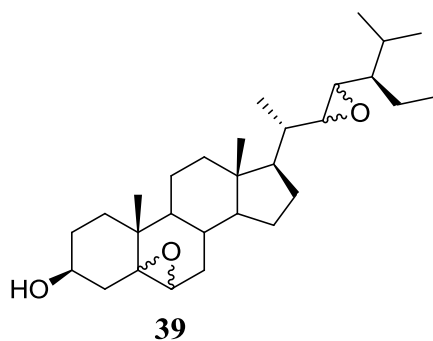


Figure 2.60 Diepoxystigmastane **39**

2.5.2 Target Molecules

Our intention was to focus on the synthesis of novel amine analogues of stigmasterol (Figure 2.61), as much data on the oxidation products of stigmasterol exists in the literature.^{34,63} This new library of compounds would be useful to compare to the lanostane series, both in terms of chemical reactivity and biological activity.

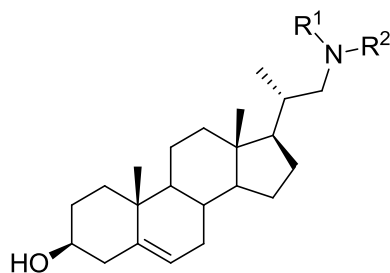


Figure 2.61

As with the lanostane series, the gateway to these amine derivatives is the side chain aldehyde **75** (Figure 2.62).

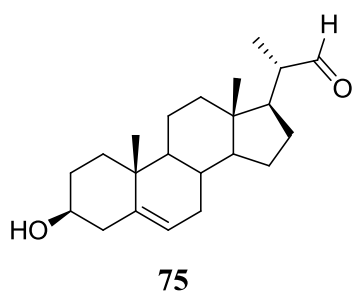
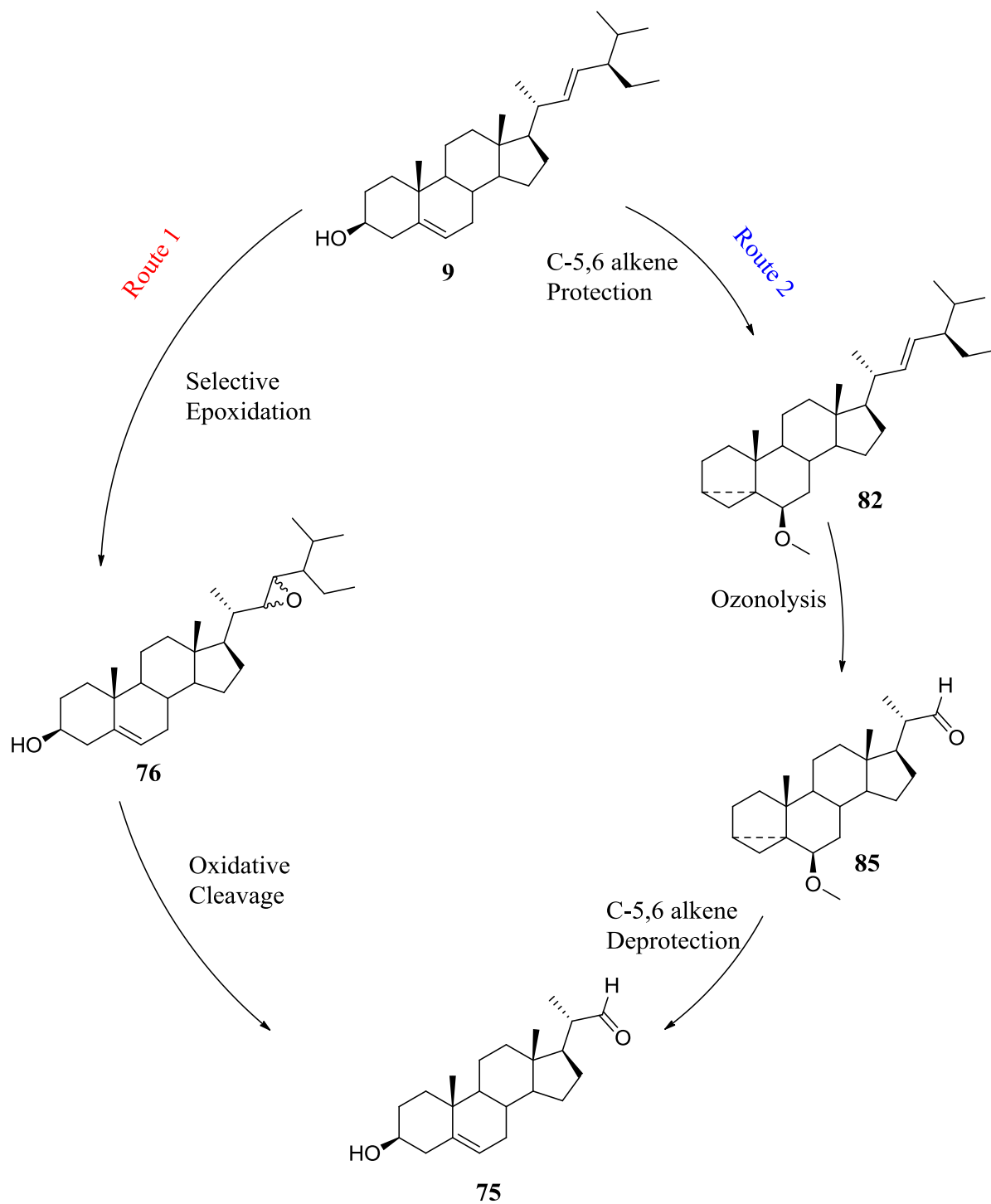


Figure 2.62

Thus, we report our strategies for the synthesis of aldehyde **75**, by both our optimised synthetic route, established for the lanostane series (Route 1), and the route documented in the literature^{7,64-66} which proceeds *via* ozonolysis (Route 2). Both of these routes use stigmasterol as the starting material for formation of the side chain aldehyde **75** (Scheme 2.54).



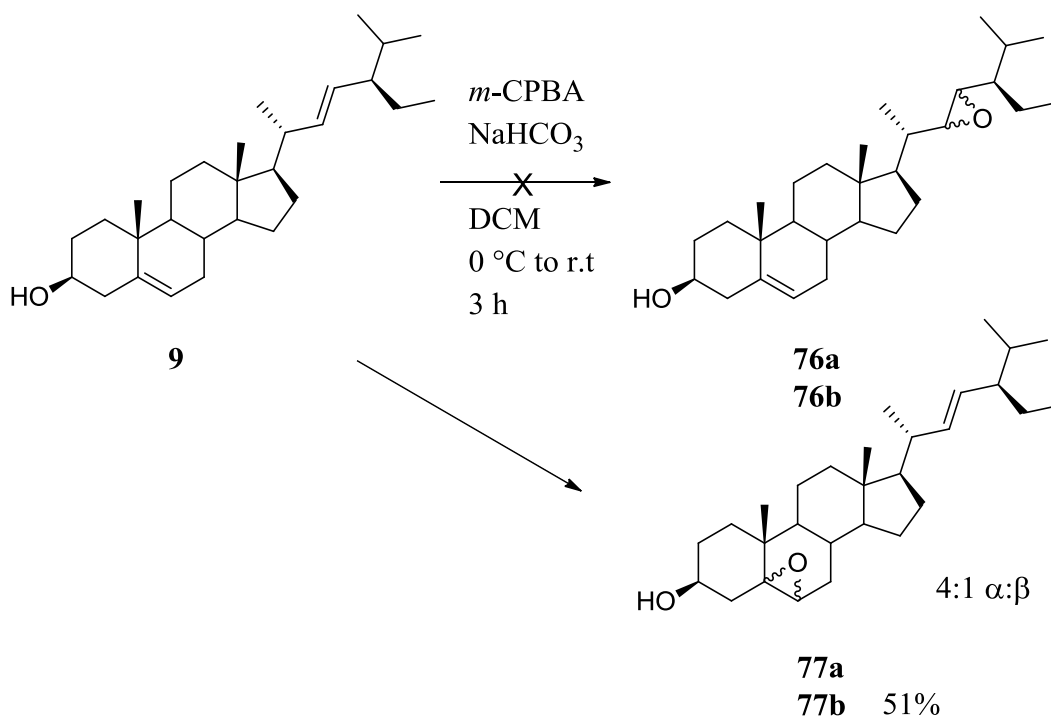
Scheme 2.54

2.5.3 Epoxidations of stigmasterol **9**

We were very keen to attempt our established oxidative cleavage methodology on the stigmasterol precursor, as we had optimised the reaction conditions and purification procedures to a high degree of efficiency in the lanostane derivative. We were also enthusiastic to avoid recourse to formation of the aldehyde by ozonolysis for several reasons. Firstly, the need to protect the C-5,6 alkene requires the two additional steps of protection and deprotection, as the ozonolysis reaction is not selective for the side chain alkene in stigmasterol. These two additional steps give rise to the possible loss of material in both the reactions and the purifications. Also, by avoiding the use of ozone, we could potentially eliminate the tedious and time consuming removal of triphenylphosphine oxide, used to decompose residual ozonide, from our product.

For the reasons outlined above, our preferred route to aldehyde **75** was Route 1. Thus we set about the test conditions outlined in Route 1, to establish if the chemistry optimised for lanosterol could be applied to the stigmasterol side chain. The aim was to selectively epoxidise the side chain alkene and subsequently cleave the epoxide to form **75**. Should this experiment be unsuccessful, we were content in the knowledge that Route 2 was available to us as an alternative method of synthesising the desired target molecule successfully.

Thus, our first reaction involved the selective epoxidation of the C-22,23 alkene by methodology effective for the lanostane derivatives. Stigmasterol **9** was reacted with *m*-CPBA and sodium bicarbonate over a three hour period in an attempt to synthesise **76** (Scheme 2.55).



Scheme 2.55

Unfortunately, instead of forming the desired product **76**, the conditions employed selectively epoxidised the C-5,6 alkene to form **77**.

Although we were hopeful that the conditions which selectively epoxidised the side chain alkene in lanosterol would reproduce a similar result in stigmasterol, this is not an unexpected result, as the C-5,6 alkene is less sterically hindered compared to that at C-22,23. It was surprising that no trace of the C-22,23 epoxide was seen in the NMR spectra of the product, and that all of the oxidant was consumed in the epoxidation of C-5,6.

The signals in the ¹H NMR spectrum which we had expected to see, should epoxidation have taken place at the C-22,23 position, were a 3H multiplet between 2.45 and 2.55 ppm corresponding to 22-CH *R* and *S* and one of 23-CH, and a 1H multiplet between 2.72 and 2.77 ppm corresponding to one of 23-CH.

Instead, very distinctive signals for compounds **77a** and **77b** in the mixture of products can be seen upon inspection of the ¹H NMR spectrum. The signal corresponding to 6-CH shows the greatest difference between the alpha and the beta isomers, and a clear difference in the chemical shifts of 3-CH for the isomers is also evident, as can be seen in Figure 2.63. The absence of the alkene hydrogen appearing as a multiplet between 5.30 – 5.37 ppm in the ¹H NMR spectrum also indicates epoxidation at the C-5,6 position.

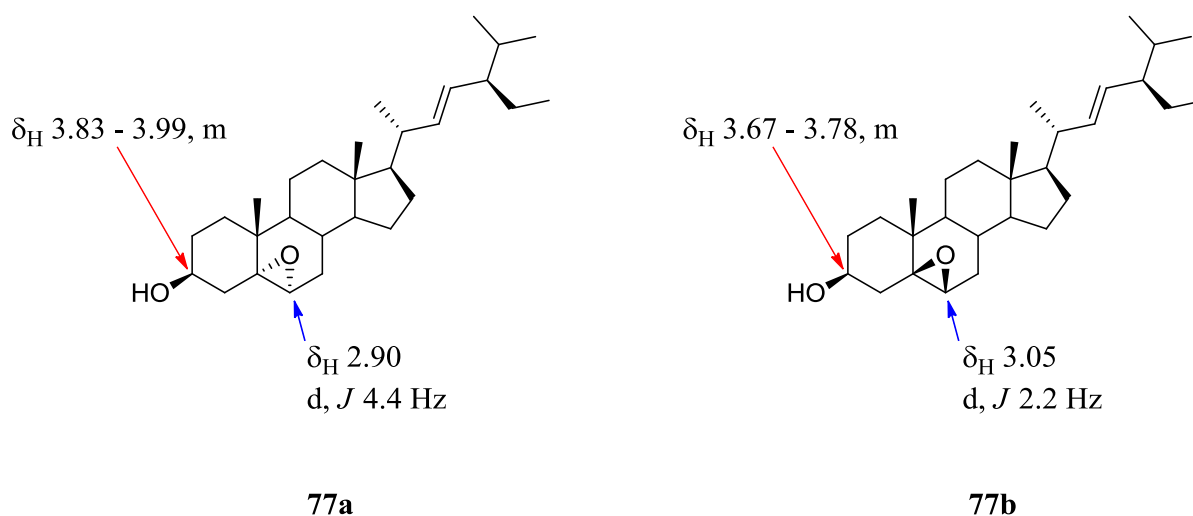
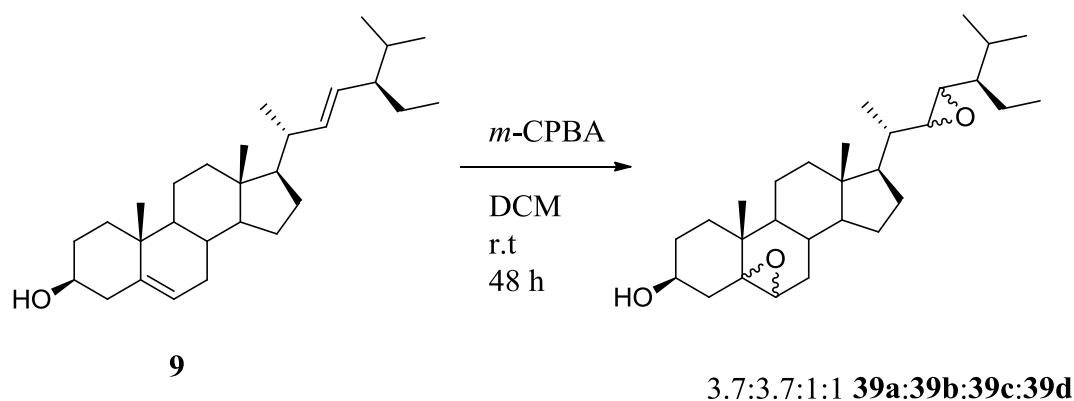


Figure 2.63

Having obtained the epoxides at the C-5,6 position, we persisted with our strategy of synthesising the aldehyde *via* oxidative cleavage of the side chain epoxide. We were optimistic that the side chain epoxide, once formed, would be selectively cleaved to yield the C-22 aldehyde, leaving the C-5,6 epoxide unreacted. Knowing that the first site of epoxidation is the C-5,6 alkene, and with this synthetic sequence in mind, stigmasterol was reacted with excess *m*-CPBA for 48 hours leading to the formation of the desired diepoxides as four isomers **39a**, **39b**, **39c** and **39d** in a 3.7:3.7:1:1 ratio (Scheme 2.56). Attempts at removal of residual *m*-CPBA by washing with sodium bicarbonate were unsuccessful. Removal of this material by column chromatography was not attempted on the grounds of potential loss of product, due to the small scale of the reaction and the limited quantity of material to work with. Therefore, we considered the subsequent test oxidative cleavage reaction of greater significance in our optimisation route.



Scheme 2.56

The C-22,23 epoxide showed overlapping signals appearing as a 3H multiplet at 2.45 – 2.55 ppm for both *R* and *S* isomers at 22-CH, and one of 23-CH, and a 1H multiplet at 2.72 – 2.77 ppm corresponded to the other 23-CH (Figure 2.64). The distinguishable signals for these 4 diastereomers are at the 6-CH position. When the C-5,6 epoxide is in the alpha position, the 6-CH proton appears as a doublet at 2.92 ppm, whereas when the C-5,6 epoxide is in the beta position, the 6-CH proton appears as a doublet at 3.08 ppm. It is the integration of these two signals which establishes the 3.7:3.7:1:1 ratio of the diastereoisomers.

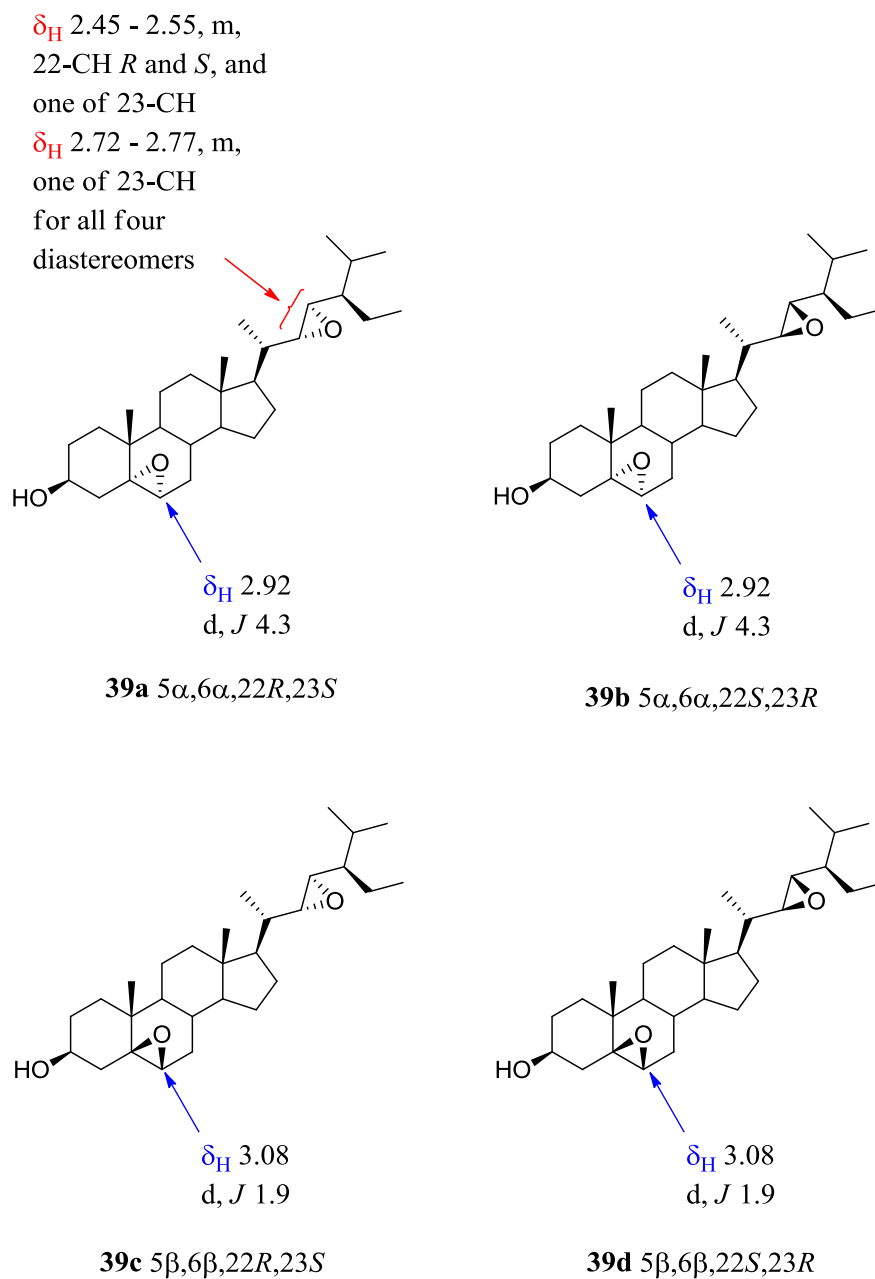


Figure 2.64

The positions and relative intensities of the pertinent peaks are seen in the ^1H NMR spectrum below,⁶⁵ where the 3.7:3.7:1:1 ratio of the **a:b:c:d** isomers of **39** can be seen clearly (Figure 2.65).

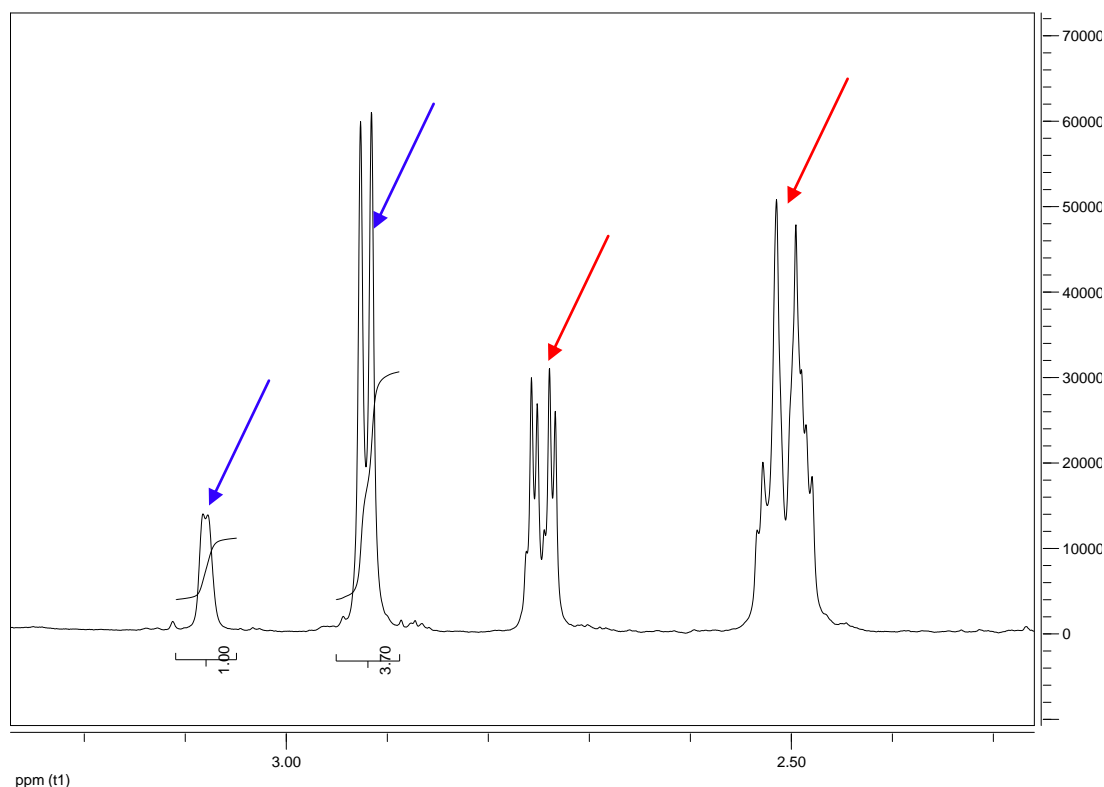
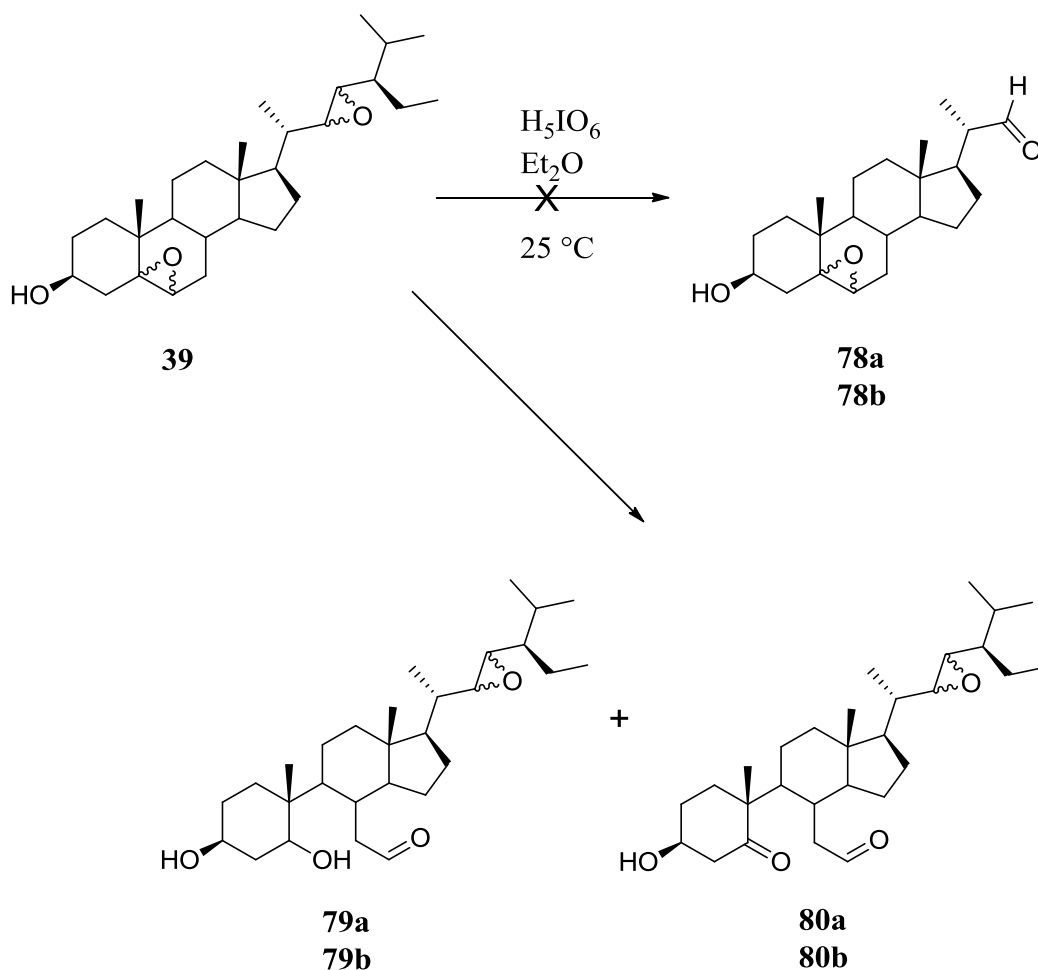


Figure 2.65: ^1H NMR spectrum showing relevant peaks of **39**

2.5.4 Oxidative Cleavage of Stigmasterol Epoxides

As discussed above, this material was carried forward in an oxidative cleavage reaction using periodic acid, with the intention of selectively cleaving the side chain epoxide to yield **78** (Scheme 2.57). However, upon inspection of the ^1H NMR spectrum of the crude material, it was seen that the side chain epoxide had not been cleaved, as the C-22,23 epoxide peaks at 2.45 – 2.55 ppm and 2.72 – 2.77 ppm were still present. It was also noted from the ^1H NMR spectrum of the crude material that the C-5,6 epoxide signals at 2.92 ppm and 3.08 ppm had disappeared, and there were new aldehyde signals in the ~ 9 ppm region. To establish the outcome of this reaction, the crude material was purified by column chromatography, leading to the isolation of two products, as shown in Scheme 2.57.



Scheme 2.57

As can be seen from Scheme 2.57, the desired product had not been formed, and unfortunately, oxidative cleavage had taken place at the C-5,6 epoxide, yielding **79** and **80**, both of which are mixtures of *R* and *S* isomers. Purification by column chromatography yielded two products, the structures of which are tentatively assigned as shown in Scheme 2.57.

In the case of **79**, an aldehyde signal was seen in the ^{13}C NMR spectrum at 204.5 ppm, and a new doublet, which we tentatively assign as 5-OH, is seen at 3.49 ppm in the ^1H NMR spectrum. The ^{13}C NMR spectrum of **80** leads us to tentatively assign its structure as shown, due to the ketone peak seen at 217.3 ppm and the aldehyde signal at 202.7 ppm.

Given that the core steroidal structure in these products had been lost due to the opening of the B ring, these products would be of no further use to us. Our biggest disappointment, however, was the fact that our synthetic strategy outlined in Route 1 (Scheme 2.54) would not be applicable to the synthesis of the key C-22 aldehyde in stigmaterol. Unfortunately, the C-

22,23 position of stigmasterol had proven much more sterically hindered than the analogous alkene in the side chain of lanosterol at C-24,25, both in terms of epoxidation and oxidative cleavage. The ethyl group at C-24 clearly has a greater effect on steric hindrance at this site than we had originally anticipated (Figure 2.66). Therefore, it was necessary to approach this chemistry from a different angle, and our revised strategy would involve proceeding to synthesise the C-22 aldehyde by Route 2 (Scheme 2.54).

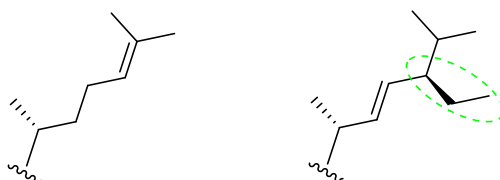


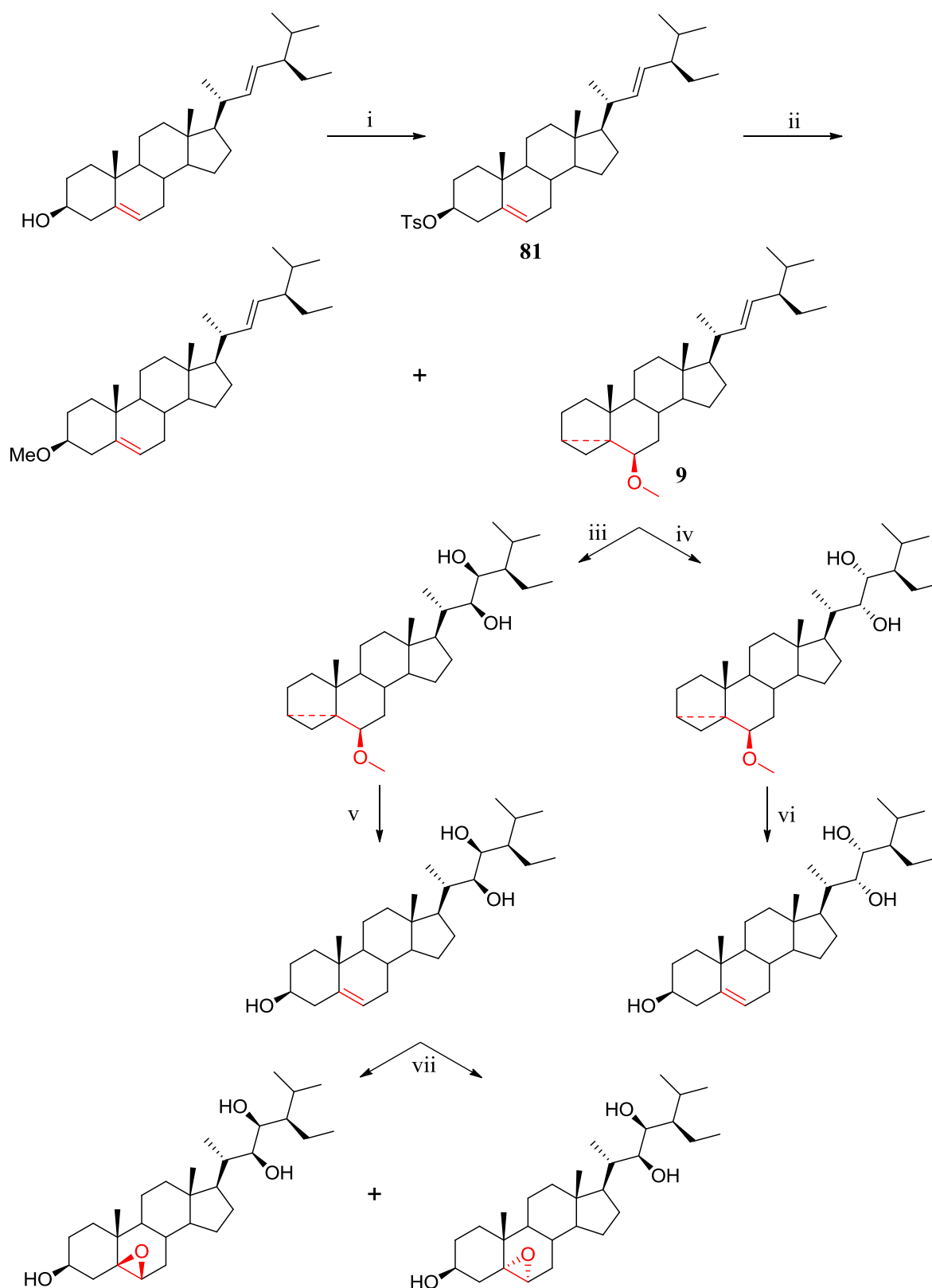
Figure 2.66: Comparison of the side chains of lanosterol and stigmasterol

2.5.5 Protection of C-5,6 alkene of stigmasterol 9 as methoxy ether

The first step in Route 2 involves the protection of the C-5,6 alkene, and the protecting group of choice was the *i*-stigmasterol methyl ether, due to its ease of formation, *via* a two step synthetic route involving tosylation and solvolysis, its stability throughout a variety of conditions, and its ease of removal using acidic reagents.⁶³

This methodology has been used extensively in cases where chemistry on the C-22,23 side chain alkene of stigmasterol requires protection of the less sterically hindered, and therefore more reactive, C-5,6 alkene.^{7,63}

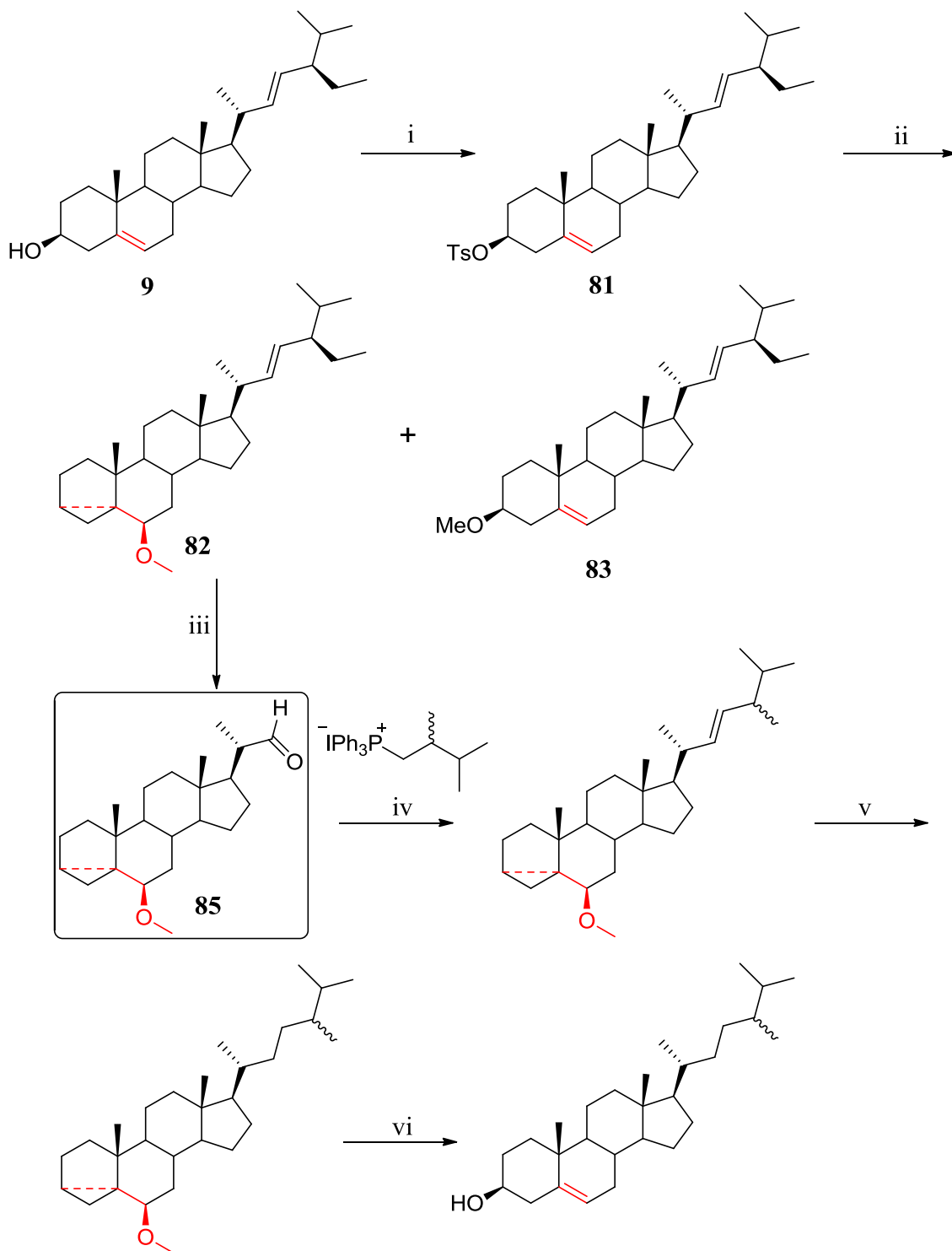
Foley *et al.* detail the protection of the C-5,6 alkene of stigmasterol by formation of *i*-stigmasterol methyl ether. Subsequent reaction of the protected steroid with osmium tetroxide under different conditions yields the 22*S*,23*S* and 22*R*,23*R* diols. These two compounds are then deprotected under acidic conditions to reform the C-5,6 alkene, which can be reacted further as shown in Scheme 2.58.⁶³



(i) *p*-TsCl, DMAP, pyridine (94%); (ii) MeOH (anhydrous), pyridine (60%); (iii) OsO₄, NMO, *t*-BuOH, THF, H₂O (47%); (iv) OsO₄, NMO, dihydroquinidine 9'-phenanthryl ether, *t*-BuOH, THF, H₂O (30%); (v) H₂SO₄, THF, H₂O (87%); (vi) H₂SO₄, THF, H₂O (67%); (vii) *m*-CPBA, DCM (45%).

Scheme 2.58

O'Connell *et al.* used the same protection strategy for the synthesis of the C-22 aldehyde and subsequent Wittig reaction (Scheme 2.59).⁷



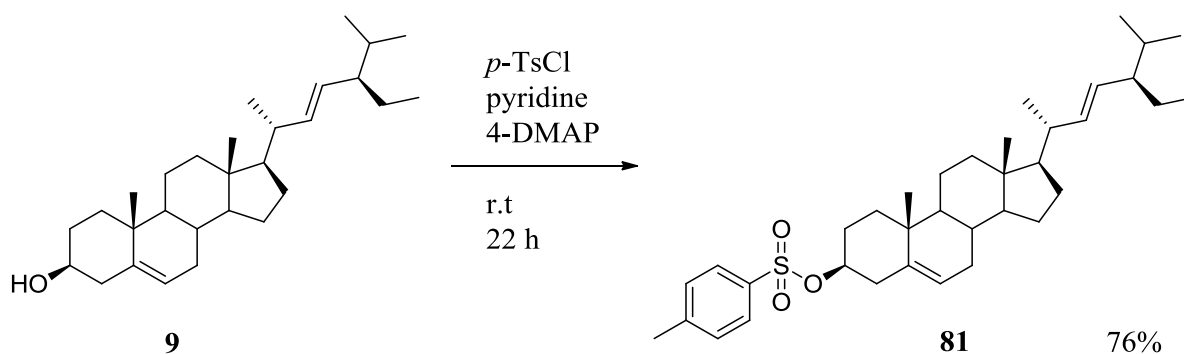
Scheme 2.59

(i) $p\text{-TsCl}$, DMAP, pyridine; (ii) MeOH (anhydrous), pyridine; (iii) O_3 ; (iv) $n\text{-BuLi}$, THF, 0 C (31%); (v) Pd/C , H_2 , EtOH, 50 psi (88%); (vi) H_2SO_4 , THF, H_2O (57%); (vii) $m\text{-CPBA}$, DCM (45%).

Note: Steps (i), (ii) and (iii) are sparsely detailed by O'Connell *et al.* and are referenced elsewhere.^{14,34,63-67}

The chemistry outlined by O'Connell *et al.* is similar to our proposed modification, in that the side chain would be cleaved to yield the C-22 aldehyde, and a new fragment added at this position. Thus we began route 2 optimistic that it would yield our desired aldehyde, given the array of literature precedent for the synthesis of this compound.^{7,14,34,63-67}

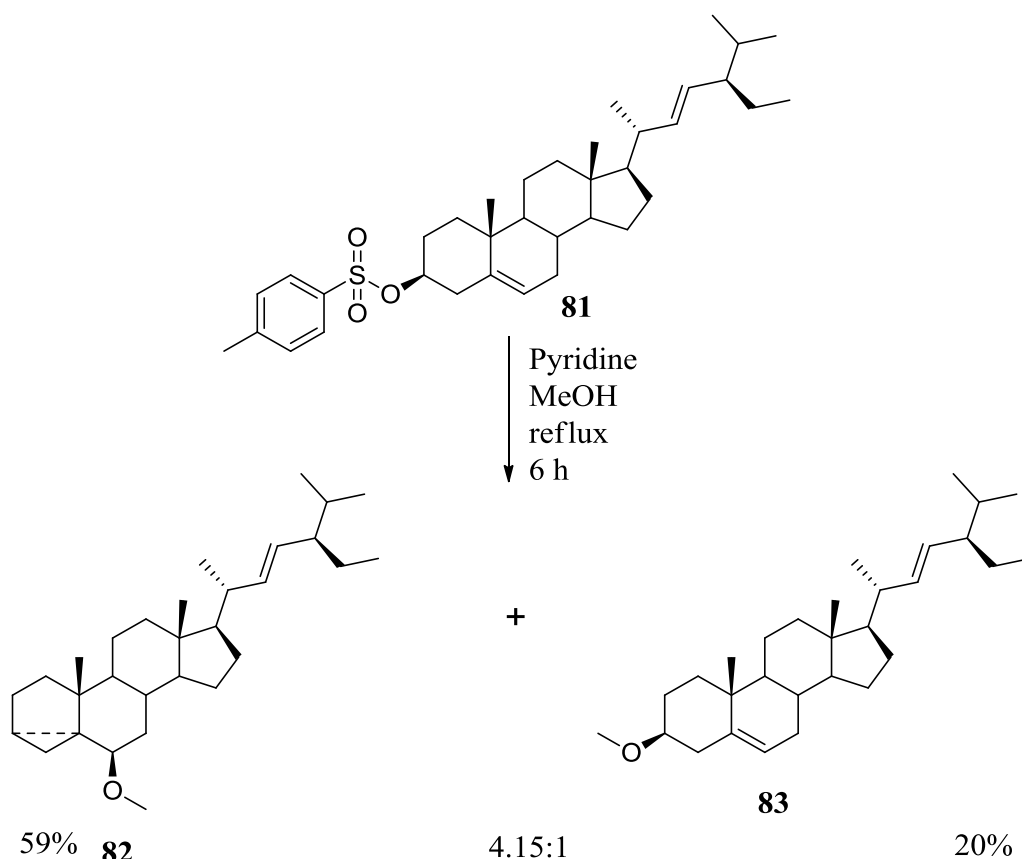
The tosylation of stigmasterol was achieved under standard conditions using *p*-toluenesulfonylchloride in pyridine, with 4-DMAP as a catalyst. The crude material was recrystallised from acetone to yield the desired tosylate **81** as a white solid in 76% yield (Scheme 2.60).



Scheme 2.60

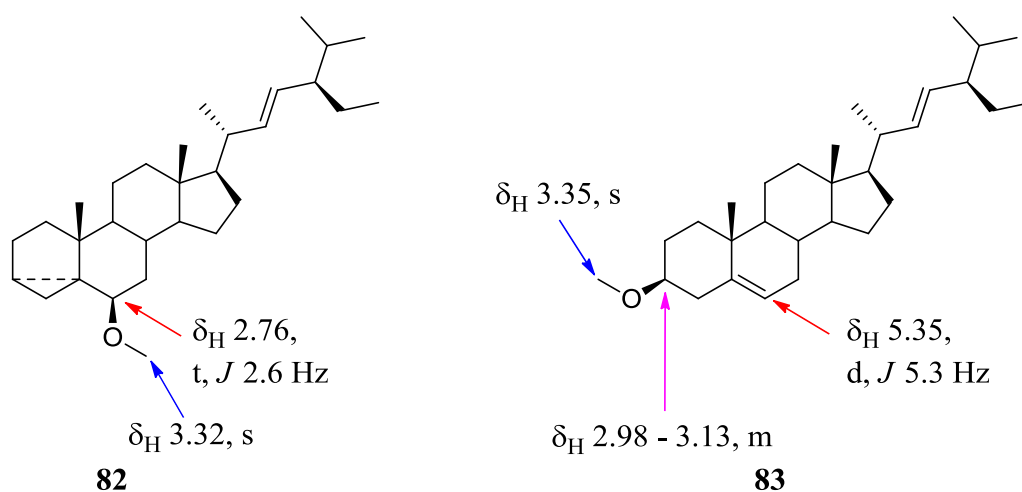
The ¹H NMR spectrum of the product shows a characteristic shift in the 3-CH signal from 3.46 – 3.57 ppm in stigmasterol to 4.24 – 4.38 ppm in the tosylate derivative as clear evidence for successful tosylation.

The tosylate **81** was carried forward to the next step of the protection which was the solvolysis reaction, a type of nucleophilic substitution or elimination where the nucleophile is a solvent molecule. This was carried out using pyridine and anhydrous methanol at reflux over a 6 hour period, leading to the formation of two products (Scheme 2.61). The desired product **82** and side product **83** were found to be formed as a 4.15:1 mixture of structural isomers, by analysis of the ¹H NMR spectrum of the mixture.



Scheme 2.61

Distinctive signals for these isomers in the ^1H NMR spectrum of the mixture are the methyl groups of the ether functionality, appearing at 3.32 ppm in **82** and 3.35 ppm in **83** (Figure 2.67). The 6-CH signal is also very distinguishing, appearing as a triplet at 2.76 ppm in **82** and an alkene signal at 5.35 ppm in **83**.

Figure 2.67: Interesting ^1H NMR differences between structural isomers **82** and **83**

Careful and repeated chromatography of these isomers yielded **82** in 59% yield and **83** in 20% yield. The formation of these isomers is by the mechanistic pathways proposed below, with the pathway leading to isomer **82** clearly being the more favourable of the two. This is due to the site of nucleophilic attack, ie C-6, being significantly less sterically hindered in this case, leading to the ~ 4:1 ratio seen in the ^1H NMR spectrum of the crude material (Figure 2.68).

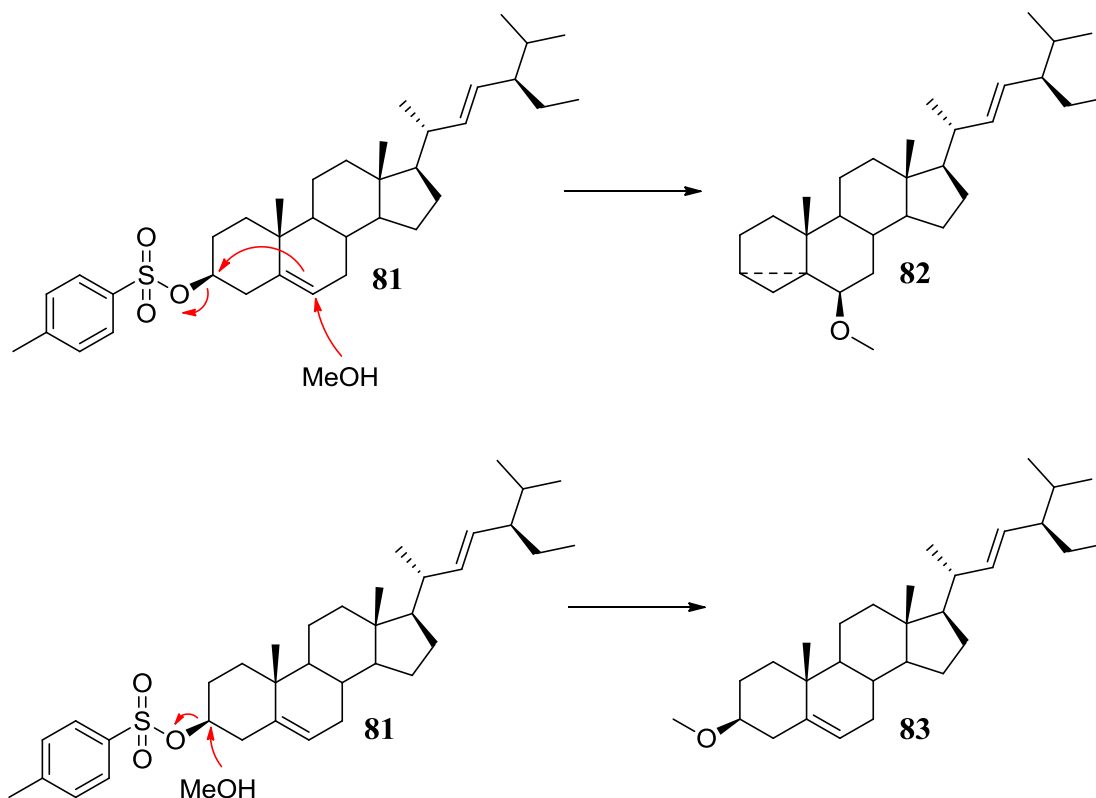


Figure 2.68

When this protected stigmasterol derivative **82** had been synthesised, we postulated that it may be possible to revert to our epoxidation and oxidative cleavage methodology for the synthesis of the C-22 aldehyde in stigmasterol. This methodology had failed in route 1 due to the fact that the C-5,6 alkene was unprotected and available for reaction. Now that we had successfully masked the C-5,6 alkene, it was worthwhile to attempt this chemistry, given the only site of reactivity would be the side chain alkene. Thus a third route, Route 3, was proposed at this point (Figure 2.69). This Route 3 is an improvement on Route 2 due to the fact that the repeated column chromatography necessary to remove triphenylphosphine oxide after the ozonolysis step would be eliminated.

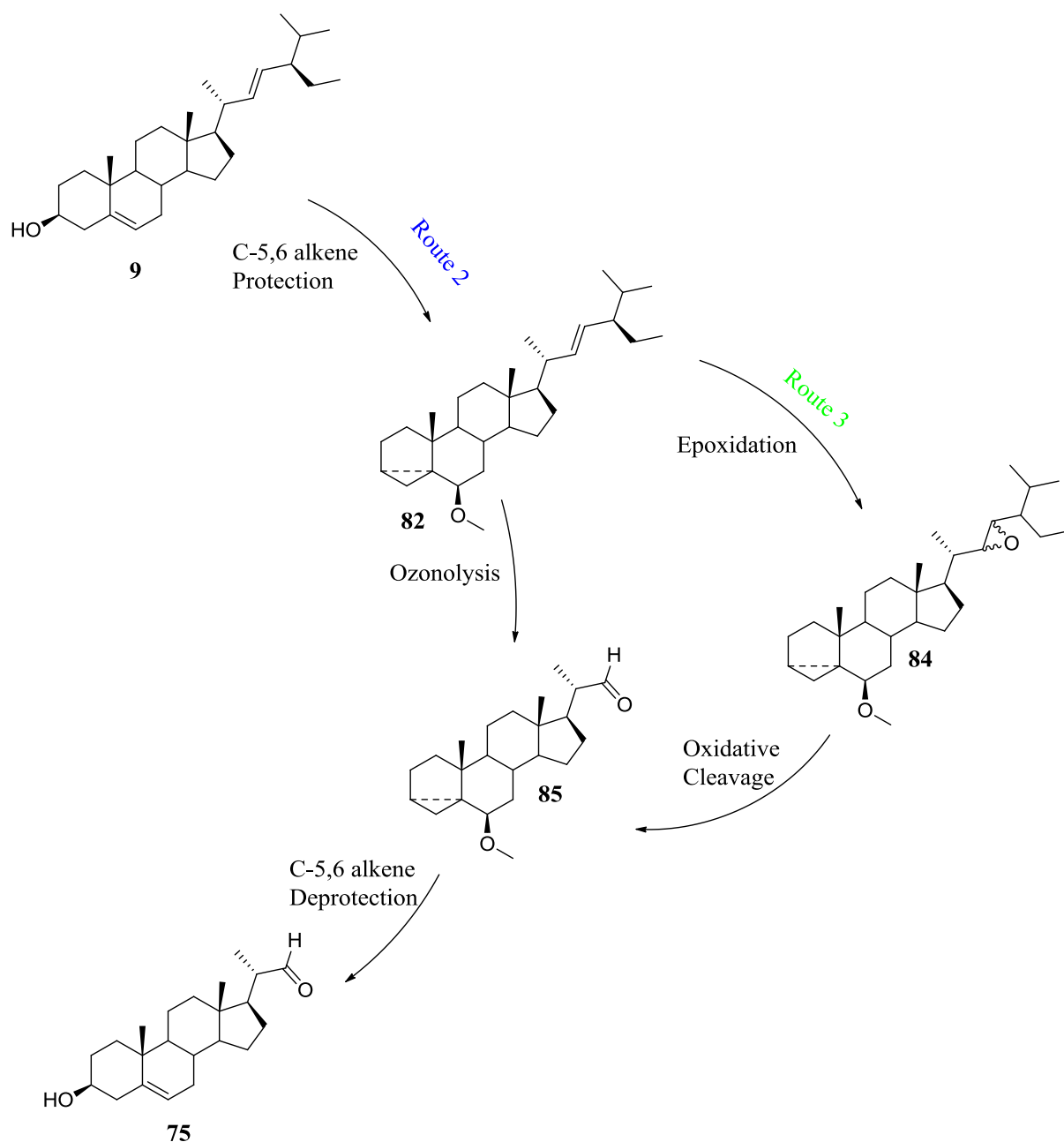
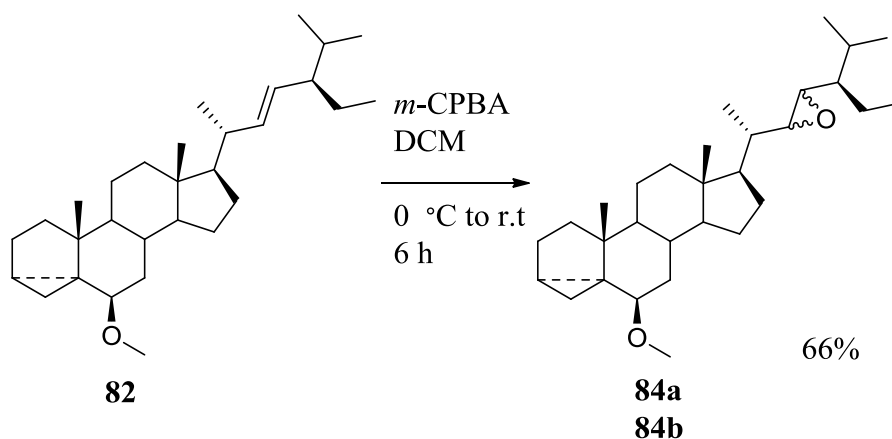


Figure 2.69

The first reaction was the epoxidation of **82**, carried out in the usual fashion with *m*-CPBA to yield **84** in 66% yield after chromatography (Scheme 2.62).

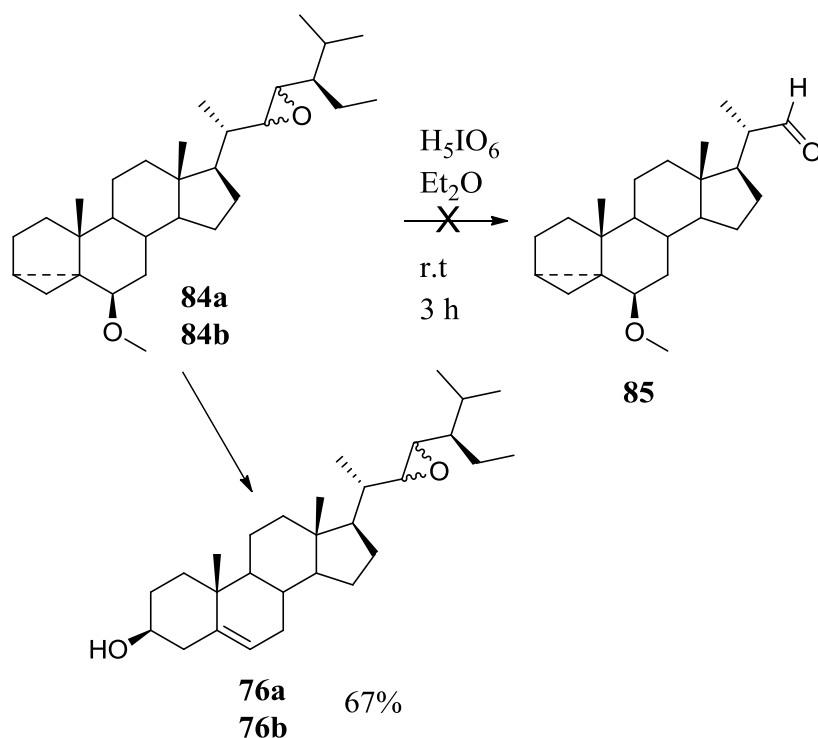


Scheme 2.62

The key signals for the C-22,23 epoxide in the ^1H NMR spectrum were similar to those seen in the previous compound with this group at this position. The *R* and *S* hydrogens at C-22, as well as 23-CH of one diastereomer appear in the multiplet at 2.43 – 2.54 ppm, while the 23-CH of the other diastereomer appears separately at 2.70 – 2.80 ppm.

2.5.6 Oxidative Cleavage of C-22,23 epoxide of stigmasterol **84**

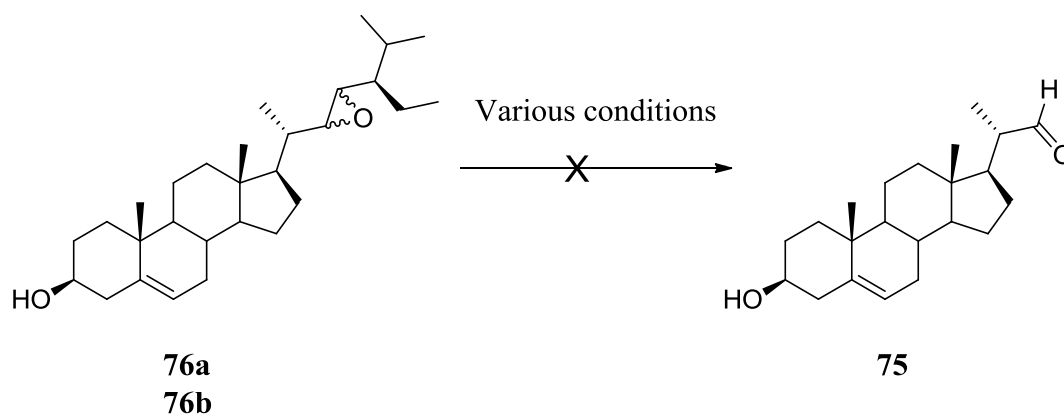
At this point, as we had now successfully synthesised the C-5,6 protected, C-22,23 epoxide, the aim was to cleave the side chain and form the aldehyde at C-22. The reaction was carried out under our conditions which were successful for the lanostane derivative, using periodic acid in diethyl ether. As we had difficulty accessing this site in previous reactions discussed above, in this instance, the reaction progress was monitored by TLC analysis until all starting material was consumed. Thus, the reaction time was increased from 10 minutes to 3 hours, in an effort to ensure complete conversion. ^1H NMR analysis of the crude material revealed that the desired aldehyde **85** had not been formed, and instead, the periodic acid had deprotected the C-5,6 alkene, yielding **76** (Scheme 2.63).



Scheme 2.63

While **76** was not the intended product of the reaction, its formation would be advantageous, if oxidative cleavage could be facilitated, as the deprotection of the C-5,6 alkene would have been undertaken in due course anyway. Therefore, this was considered an opportunity to optimise suitable conditions for the formation of the C-22 aldehyde and deprotection the C-5,6 alkene in one pot. The conditions attempted for the oxidative cleavage of the C-22,23 epoxide and synthesis of **75** are detailed in Table 2.17.

Table 2.17



Entry	Reagent	Equivalents	Temp	Time	Solvent	Reaction Outcome
1	H ₅ IO ₆	6	reflux	6 h	Et ₂ O	No evidence of product
2	NaIO ₄	2	r.t	6 h	2:1 THF:H ₂ O	No evidence of product
3	NaIO ₄	4	r.t	6 h	4:1 AcCN:H ₂ O	No evidence of product

As can be seen in Table 2.17, there was no evidence of product formation after multiple attempts at oxidative cleavage.

It was our belief that the chemistry, which was so successful for the lanostane side chain, could be applied in some way to the relatively similar side chain of stigmasterol. However, it has transpired that the presence of an ethyl group at C-24 in stigmasterol has an adverse effect in terms of steric hinderance and completely blocks the oxidative cleavage at the site of interest, regardless of the protection of other vulnerable functionalities.

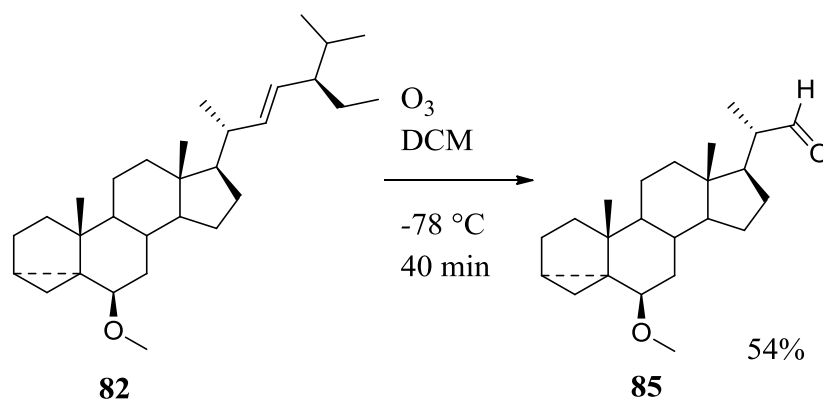
Due to the fact that Routes 1 and 3 had failed, we reverted to Route 2 for the synthesis of the C-22 aldehyde of stigmasterol, which had been reported in the literature.⁶³

2.5.7 Ozonolysis of protected stigmasterol **82**

Ozone gas is the reagent for this process, and is prepared by passing a stream of oxygen through a high voltage electrical discharge. At low temperatures, ozone adds rapidly to an alkene to form a highly reactive species known as an ozonide. The ozonide then breaks down to form two carbonyl moieties where the carbon carbon double bond had been.

This procedure was undertaken on our protected C-5,6 alkene **82**, as the ozonolysis reaction has no selectivity for specific double bonds. Prior solvent studies were carried out to ensure the substrate would remain in the chosen solvent at -78°C . Dichloromethane emerged as the most suitable solvent for reaction at this temperature, as none of the substrate was observed to precipitate at -78°C . This low temperature is necessary to prevent detonation of the reactive ozonide.

The reaction proceeded well, yielding the desired compound **85** as a white solid in 54% yield following multiple purifications *via* column chromatography (Scheme 2.64).



Scheme 2.64

Repeated column chromatography was necessary to remove excess triphenylphosphine oxide present in the crude product. In fact, the triphenylphosphine oxide was present at such a level that the steroidal signals were completely diminished in the ^1H NMR spectrum of the crude product. This presence of triphenylphosphine oxide is due to the addition of a vast excess of triphenylphosphine to the reaction mixture upon completion of the ozonolysis, in order to remove residual ozonide.

A pure sample of **85** was ultimately obtained in 54% yield. Though this yield may seem moderate, it is in fact quite satisfactory for a reaction of this type, given the repeated and tedious chromatography involved. Aldehyde **85** was characterised by ^1H and ^{13}C NMR spectroscopy, showing very distinctive signals at 9.58 ppm in the ^1H NMR spectrum and 205.3 ppm in the ^{13}C NMR spectrum. It was also important to note that the protecting group had been retained, given the presence of the singlet at 3.33 ppm in the ^1H NMR spectrum and the signal at 56.6 ppm in the ^{13}C NMR spectrum (Figure 2.70).

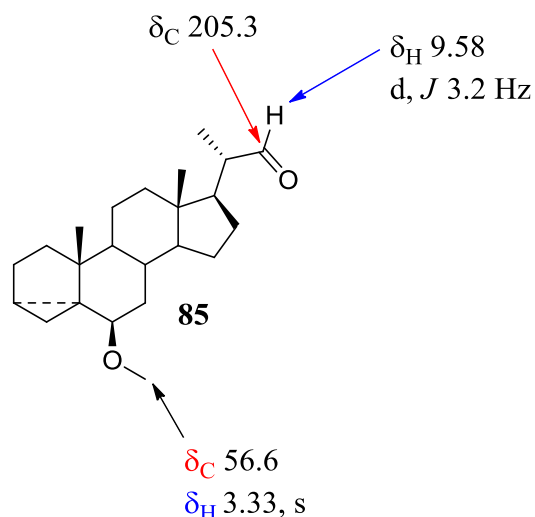


Figure 2.70

We were delighted with the successful synthesis of the C-22 aldehyde, following much work in this area. Moreover, the compound was synthesised in such a high level of purity that we were content to proceed with reductive aminations on this aldehyde.

2.5.8 Amine Derivatives of Stigmasterol

Thus our target molecules emerged as the stigmasterol nucleus with amine side chains (Figure 2.71), which would provide a valuable comparison with the side chain amines on the lanostane nucleus, both in terms of the chemical reactivity towards reductive amination, and their biological activity against U937 cells.

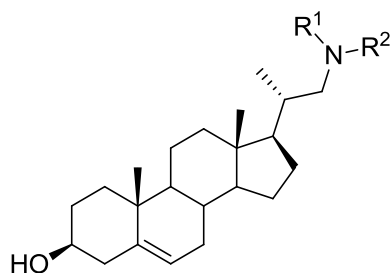
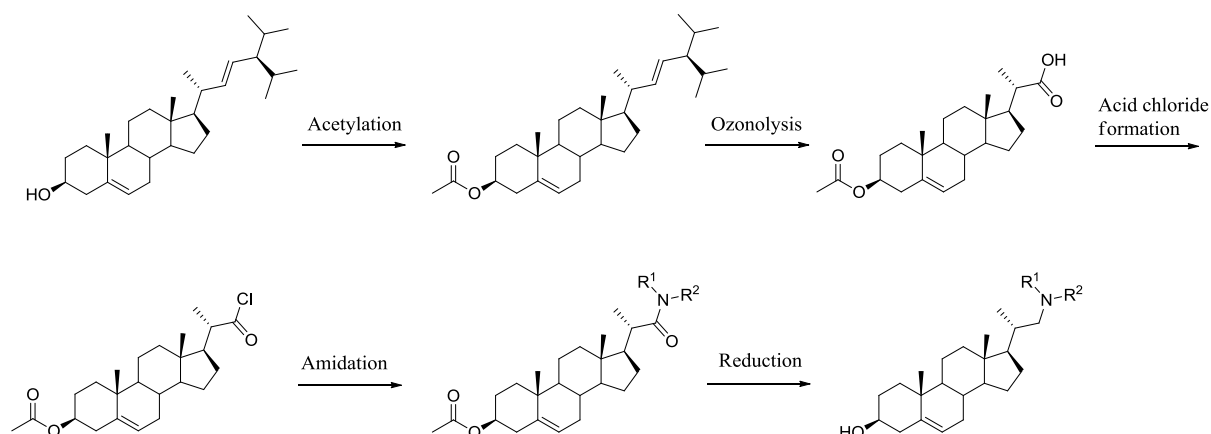


Figure 2.71

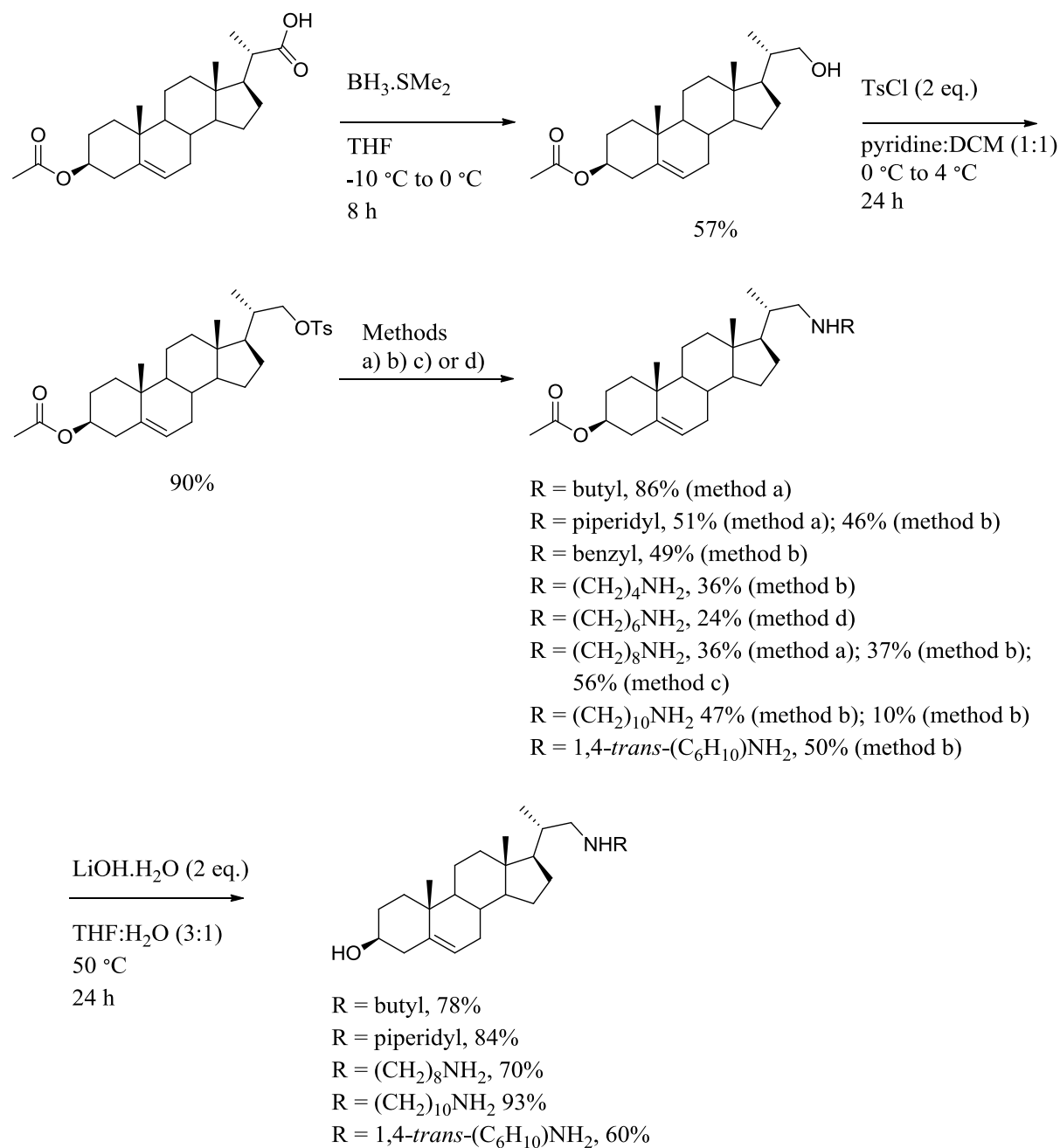
There have been reports of the synthesis of amine derivatives of the stigmasterol nucleus in the literature by Herzog *et al.* in 1955 and Lorente *et al.* in 2005.^{36,37} These compounds were formed by multi step syntheses in both cases and biologically evaluated for antifungal activity and enzyme inhibition.

Herzog *et al.*³⁶ report the synthesis of amine derivatives starting from stigmasterol. The amines were synthesised *via* a five step route (Scheme 2.65).



Scheme 2.65

Lorente *et al.*³⁷ describe an alternative route for formation of amines in the side chain of the stigmasterol nucleus, starting from the commercially available sterol 3 β -acetoxy-5-cholenic acid. This acid was reduced to the alcohol using borane-dimethyl sulfide, which was selective for the carboxylic acid functionality and did not affect the acetate group or the double bond in the sterol.^{68,69} The alcohol was then tosylated,⁷⁰ and displacement of the tosyl group by four different methods led to the synthesis of a library of amines, as shown in Scheme 2.66. The free hydroxyl analogues of a selection of these compounds were prepared using lithium hydroxide.⁷¹

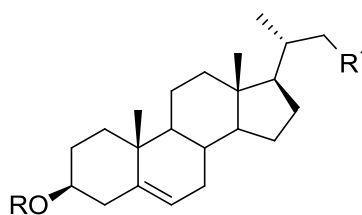


Reagents and conditions: (Method a) amine (10 – 20 eq.), diisopropylethylamine (3 eq.), DMF, $60\text{ }^\circ\text{C}$, 48h; (Method b) Amine (3 – 4 eq.), K_2CO_3 (1.5 eq.), $\text{Bu}_4\text{N}^+\text{T}^-$ (cat.), DMF, $100\text{ }^\circ\text{C}$, 4 h; (Method c) Diamine (4 eq.), EtOH (40 mL), reflux; (Method d) Diamine (4 eq.), EtOH (30 mL), DMF (20 mL) reflux.

Scheme 2.66

These compounds were evaluated against the recombinant *L. major* 24-SMT. The enzyme was over expressed in *E. coli*, and the enzyme assays were conducted using *E. coli* cell-free extracts containing soluble protein as the enzyme source. The results of the study conducted by Lorente *et al.*³⁷ are displayed in Table 2.18.

Table 2.18



Entry	R	R ¹	IC ₅₀ (μM) <i>L. major</i> 24-SMT
1	Ac	NHbutyl	> 100
2	Ac	Piperidyl	> 100
3	Ac	NHCH ₂ Ph	> 100
4	Ac	NH(CH ₂) ₄ NH ₂	16
5	Ac	NH(CH ₂) ₆ NH ₂	
6	Ac	NH(CH ₂) ₈ NH ₂	35
7	Ac	NH(CH ₂) ₁₀ NH ₂	
8	Ac	<i>Trans</i> -1,4-C ₆ H ₁₀ NH ₂	> 100
9	H	NHbutyl	0.97
10	H	Piperidyl	6.4
11	H	NH(CH ₂) ₈ NH ₂	2
12	H	NH(CH ₂) ₁₀ NH ₂	
13	H	<i>Trans</i> -1,4-C ₆ H ₁₀ NH ₂	45

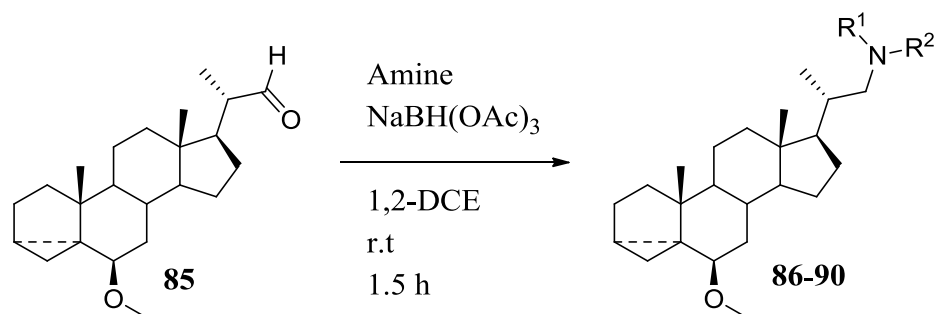
It has been proposed by Nes⁷² that inhibitors of 24-SMT must have a free 3β-OH for binding to the active site. However, the compounds protected with an acetate at the 3β position were also assayed for activity, as Lorente envisaged the acetate group being cleaved *in vitro* or *in vivo*.³⁷ Upon biological evaluation of these amines for enzyme inhibition, it was found that the proposal by Nes was proven accurate, as the OH protected analogues showed little or no activity. However, compounds with free alcohol functionality at C-3 exhibited good inhibition of the enzyme, as can be seen in Table 2.18.

With the knowledge of the work done in this area we set about the synthesis of such derivatives, with the intention of having these compounds biologically evaluated for anti cancer activity, which to our knowledge, has not been previously reported.

2.5.9 Reductive aminations of 20(*S*)-methyl-6(*R*)-methoxy-3,5-cyclo-pregnane-21-al **85**

Our target molecules emerged as the morpholine, piperidine, thiomorpholine, 1-(2-hydroxyethyl) piperazine and diethylamine derivatives. These specific amines were chosen for reductive amination based on the success of their analogous lanostane derivatives, from a chemical or biological point of view. The morpholine and thiomorpholine derivatives were unproblematic in terms of chemical reactivity in our lanostane series, and gave moderate to good yields. Whereas the piperidine, 1-(2-hydroxyethyl) piperazine, and diethylamine derivatives were successful in terms of both a chemical and biological point of view, giving good yields and excellent biological activity. Thus, we synthesised a range of novel amine derivatives of our protected stigmasterol (Table 2.19).

Table 2.19



Entry	Amine	Product	Compound	Conversion	Isolated Yield
1			86	60%	43%
2			87	50%	13%
3			88	80%	29%
4			89	>95%	31%
5			90	-	-*

* Upon inspection of the ^1H NMR spectrum there was no evidence of any consumption of starting material or conversion to product

It can be immediately seen from Table 2.19 that the yields of these compounds were significantly lower than their corresponding lanostane derivatives. This is explicable by the lower percentage conversions of these reactions as discussed below.

2.5.9.1 Reductive amination of **85** with morpholine

The first novel derivative, **86**, was synthesised by reductive amination of aldehyde **85** with morpholine (Table 2.19, entry **1**). ^1H NMR analysis of the crude material showed the reaction had gone 60% to completion, with ~ 40% of aldehyde **85** remaining, therefore, it was necessary to purify the product. Initially, we were reluctant to resort to column chromatography, as our previous steroidal amines had been incompatible with this purification technique. In the case where separation of the minor regioisomer of the 1-(2-aminoethyl) derivative in the lanostane series was necessary, column chromatography using hexane and ethyl acetate led to the complete loss of product. However, given the quantity of aldehyde starting material present in the crude product from this reaction, it was necessary to revise our purification strategy. Several test TLC eluents were employed to establish the R_f values of the product in different solvents, and we concluded that an 85:15 mixture of ethyl acetate and ether was the optimum solvent system for column chromatography of this product. Therefore a very fast column was undertaken, with the desired product eluting in very early fractions. It is possible that the shorter alkyl side chain in the stigmasterol analogue of the amine renders the compound slightly less polar and allows successful column chromatography when a highly non-polar solvent system is used. Thus, the morpholine derivative **86** was recovered in 43% yield following column chromatography.

We were satisfied with the successful purification of this derivative to remove the starting aldehyde material from the crude product, and were optimistic that the same purification strategy could be applied going forward, should starting material remain after future reactions of this type. It is likely that this incomplete conversion of aldehyde to amine is attributable to the site of reactivity now being in much closer proximity to the steroidal rings and C-21, causing increased steric hindrance to an approaching nucleophile (Figure 2.72).

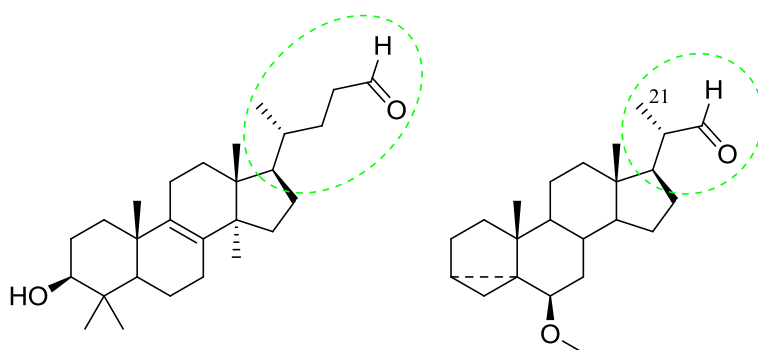


Figure 2.72

The ^1H NMR spectrum of the purified product, **86**, shows the expected characteristic disappearance of the 22-CH aldehyde signal at ~ 9.5 ppm. This new signal, corresponding to 22- CH_2 , shows an intricate splitting pattern. One hydrogen of the methylene group appears as a doublet of triplets at 2.02 ppm, while the other hydrogen is contained within a multiplet between 2.14 and 2.32 ppm. It will transpire, upon further discussion, that this is a common occurrence across the series of stigmastane amine derivatives. In fact, the methylene hydrogens, contained within the multiplet in this case, become distinctive in the thiomorpholine derivative when nearby protons display a different chemical shift.

Returning to analysis of the ^1H NMR spectrum of **86**, another key characteristic of successful reductive amination is that the stereogenic 20-CH, originally adjacent to a carbonyl, is now shifted upfield from a multiplet between 2.32 – 2.45 ppm to within the large 40H multiplet accounting for the majority of the steroidal signals. The very distinctive 4H multiplet between 3.60 and 3.75 ppm corresponding to the two morpholine O- CH_2 groups is visible in this spectrum also, in a comparable region to previous morpholine derivatives (Figure 2.73).

The absence of the carbonyl peak at 205.3 ppm in the ^{13}C NMR spectrum substantiates successful reductive amination, however the new C-22 signal is indecipherable between 54.3 and 65.2 ppm. This CH_2 group adjacent to the new nitrogen moiety previously appeared at ~ 60 ppm in our lanosterol derivatives, however, in the stigmastane derivatives it is directly adjacent to a stereogenic centre, which has an unknown effect on the chemical shift value. A distinctive peak in the ^{13}C NMR spectrum is that at 67.2 ppm, corresponding to the morpholine O- CH_2 groups.

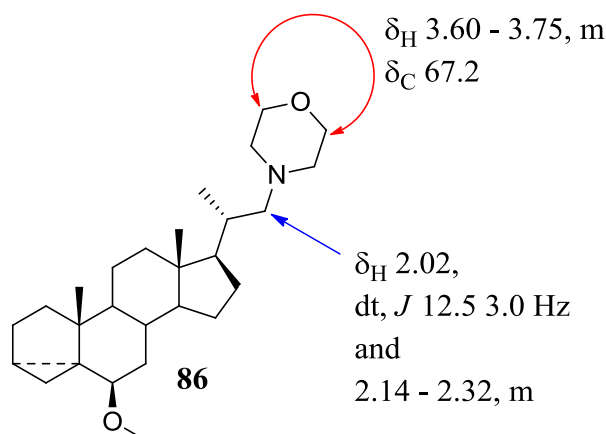


Figure 2.73

This compound is awaiting evaluation for cytotoxicity and has been fully characterised and stored in preservation for possible future acceptance for biological screening.

2.5.9.2 Reductive amination of **85** with piperidine

Piperidine derivative **87** (Table 2.19, entry **2**) was formed in 50% conversion, however isolated in particularly low yield of 13%. Upon reaction completion, inspection of the ^1H NMR spectrum of the crude material showed only 50% conversion of aldehyde to amine was achieved. There had been no change in the reaction conditions for the synthesis of this derivative, as we had established a successful purification technique for these compounds. When column chromatography was performed, using 85:15 ethyl acetate:ether only the analytically pure fractions were combined and characterised, leading to the poor yield of 13%. By comparison with morpholine derivative, (Table 2.19, entry **1**) the piperidine derivative (Table 2.19, entry **2**), it is evident that the reductive amination involving piperidine was less successful than that of morpholine, both in terms of percentage conversion and yield. This is a surprising result, as no issues arose with piperidine in the lanostane series of amines.

The ^1H NMR spectrum of **87** was much the same as that of **86** in terms of characteristic shifts of key signals. The interesting appearance of the 22- CH_2 hydrogens separately, as a doublet of triplets at 2.01 ppm and within a multiplet between 2.09 and 2.28 ppm, is evident again in this derivative (Figure 2.74). In the ^{13}C NMR spectrum, it is not clear where the 22- CH_2

signal appears, as there is no distinctive methylene signal in the 60 ppm region. As with the morpholine derivative, the C-22 signal is indecipherable between two possibilities. HRMS shows the presence of the desired product, as an exact mass of 414.3729 was found, correlating closely with a calculated mass of 414.3736.

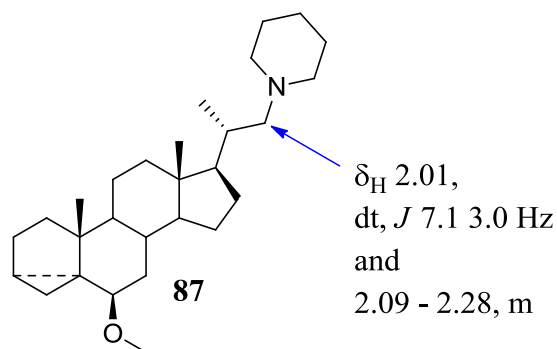


Figure 2.74

2.5.9.3 Reductive amination of 85 with thiomorpholine

Thiomorpholine derivative **88** (Table 2.19, entry **3**) was synthesised in 80% conversion and 29% yield following reductive amination. This is an encouragingly high percentage conversion to the desired product, however, the repeated column chromatography required to obtain an analytically pure sample significantly lowered the percentage yield.

As discussed earlier, the ^1H NMR spectrum of **88** clearly shows the two hydrogens of 22- CH_2 as separate peaks occurring as a doublet of triplets at 2.01 ppm, and a doublet of doublets at 2.31 ppm (Figure 2.75). The more downfield of these signals, at 2.31 ppm, previously obscured by the overlapping N- CH_2 signal in other derivatives, is now clearly visible. This is explained by the N- CH_2 signal shifting further downfield, due to the presence of the nearby sulfur heteroatom, and now being contained within the 2.40 – 2.72 ppm multiplet.

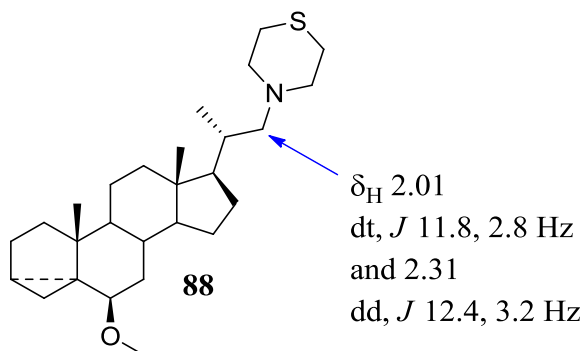


Figure 2.75

Indeed, it was the assignment of **88** which gave us the insight into the unusual splitting pattern the methylene group at C-22, adjacent to a stereogenic centre. It is the diastereotopicity of these protons which leads to their different chemical shifts and unusual multiplicities.

This novel compound was fully characterised and preserved for possible future biological testing.

2.5.9.4 Reductive amination of **85** with 1-(2-hydroxyethyl)piperazine

Given the excellent biological activity of the 1-(2-hydroxyethyl)piperazine derivative in our lanostane series, we were enthusiastic about the stigmastane analogue. Compound **89** (Table 2.19, entry **4**) was synthesised in 31% yield. Interestingly, ^1H NMR analysis of the crude material showed complete conversion of aldehyde starting material to product **89**, as there was no signal observed in the 9.5 ppm region. Column chromatography was not required in this case, as integration of the characteristic peaks in the ^1H NMR spectrum showed the product was analytically pure. The poor yield of this material is disappointing, however, the reaction was only carried out on one occasion. This reaction will need to be repeated in future work to establish the reproducible yield.

This is a surprising result, as previous derivatives showed incomplete conversion of starting material to product in varying degrees. It would be expected that incomplete conversion would occur in this case also, possibly to an even higher extent as a more sterically bulky amine is being used, however this was not the observed case.

The poor yield for the 1-(2-hydroxyethyl)amine derivative of stigmasterol cannot be attributed to a poor nucleophilic effect of the amine, as previous reductive aminations using this amine showed very good yield in the lanostane derivatives. Yields of 94%, 88% and 72% were achieved for the acetate, alcohol and ketone derivatives of the lanostane nucleus upon reductive amination with this amine.

The ^1H NMR spectrum of the product did not show the key 22- CH_2 signals as distinctly as the thiomorpholine derivative. In fact, the signals corresponding to these two hydrogens were contained within separate multiplets at 0.77 – 2.08 ppm and 2.10 – 2.40 ppm. The CH_2 group adjacent to the alcohol in the hydroxyethyl side chain can be clearly seen as a distinctive triplet at 3.61 ppm, while three of the N- CH_2 groups are contained within a multiplet at 2.45 – 2.70 ppm.

Again, this novel compound was fully characterised and preserved for future biological evaluation.

2.5.9.5 Reductive amination of **85** with diethylamine

The remaining target molecule to be synthesised in this series was the diethylamine derivative **90** (Table 2.19, entry **5**). Identical conditions of temperature and time were employed for the synthesis of this derivative, however, upon reaction completion and work up, analysis of the ^1H NMR spectrum of the crude material showed only starting aldehyde **85** was present, with no indication of formation of the desired product.

This complete non-conversion of starting material to product was a somewhat unexpected result. As discussed previously, the conformationally unrestricted nature of the diethyl groups was envisaged to have an impact on reductive amination, as the rotation of these groups could potentially block the nitrogen lone pair from acting in a nucleophilic reaction. However, in our previously synthesised lanostane analogues, this issue was not problematic.

The reaction was repeated using the crude material from the first attempt and increasing the reaction time to 24 hours. Again there was no evidence of the formation of the desired product and the reaction was not pursued further.

It is postulated that the free rotation of the ethyl groups, coupled with the proximity of the aldehyde to the steroidal ring and the C-21 methyl group, creates an insurmountable barrier to

reaction on steric grounds (Figure 2.76). While this effect was not seen in the lanostane derivatives, it becomes relevant in this case due to the proximity of the C-21 methyl group to the site of reactivity, blocking the attack of the already hindered nitrogen lone pair.

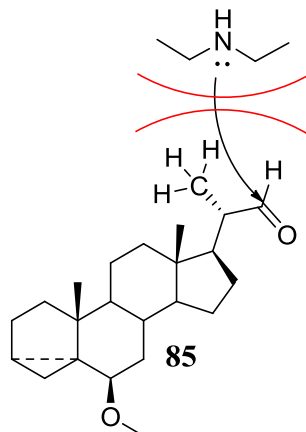
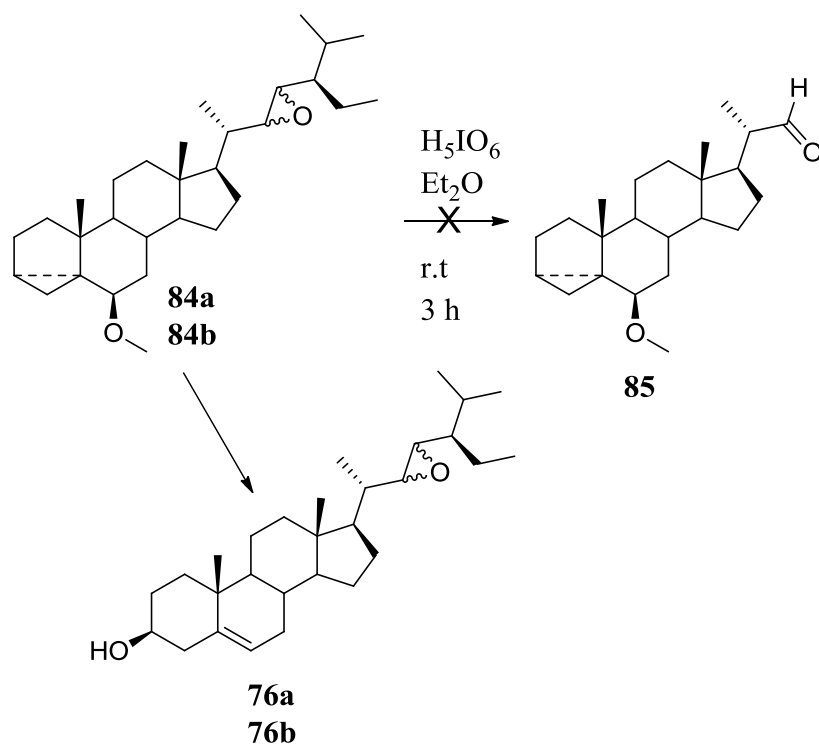


Figure 2.76

In summary, with the exception of the diethylamine derivative, these reductive aminations proceeded with moderate to excellent percentage conversions (50% - \geq 95%). It is unfortunate that purification of these compounds required repeated column chromatography, resulting in lower yields than their corresponding lanostane derivatives. However, the synthesis of these compounds provides a starting point for further research in this area, which would hopefully lead to better yields and an increase in the library of these amines with a cholestane nucleus.

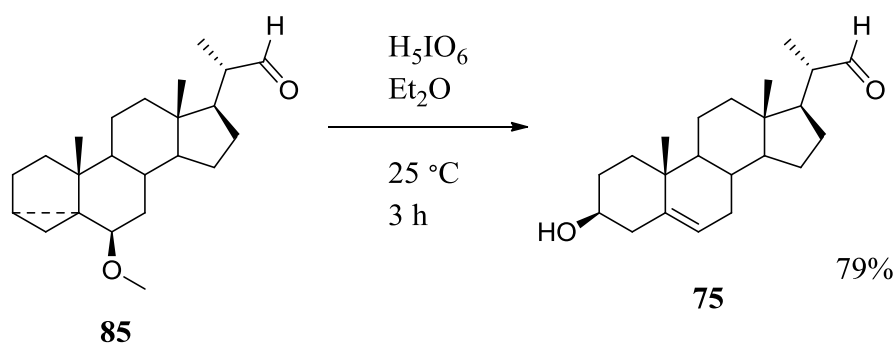
2.5.10 Deprotection of C-5,6 alkene

In a previous reaction in Route 3, Scheme 2.67, we had inadvertently deprotected the C-5,6 alkene of **84**, in a reaction that was intended to cleave the C-22,23 epoxide, using periodic acid.



Scheme 2.67

We therefore employed these conditions to deprotect aldehyde **85**, which was formed by ozonolysis. Although the deprotection conditions used by Foley *et al.*, namely sulfuric acid in water and THF,⁶³ were not employed in this case, our acidic deprotection yielded **75** as an off white solid in 79% yield (Scheme 2.68). The periodic acid was considered a better reagent than sulfuric acid for this deprotection as it led to an improvement on the 67% yield reported by Foley.



Scheme 2.68

Clear evidence for deprotection of the C-5,6 alkene was seen by the re-appearance of key signals in the ^1H NMR spectrum of the product (Figure 2.77). The alkene hydrogen 6-CH appeared at 5.35 ppm, while 3-CH appeared as a multiplet between 3.46 and 3.59 ppm, both in their original regions as seen in the stigmasterol starting material. The absence of the 3H singlet at 3.33 ppm further demonstrated that the methyl ether was no longer present at C-6. The ^{13}C NMR spectrum of the product is indicative of successful deprotection also, showing the re-emergence of the alkene signal at 120.3 ppm and the C-3 signal at 70.7 ppm.

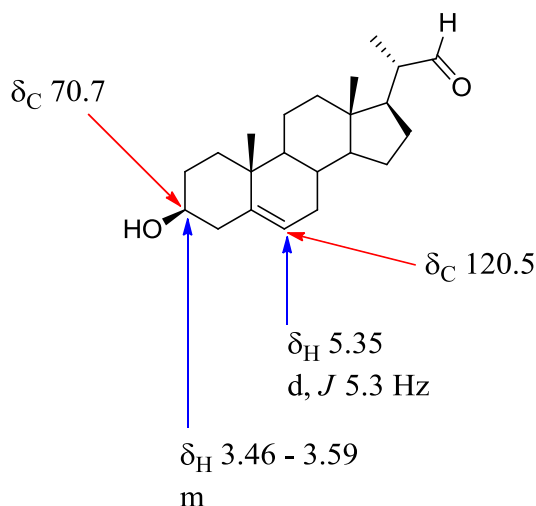
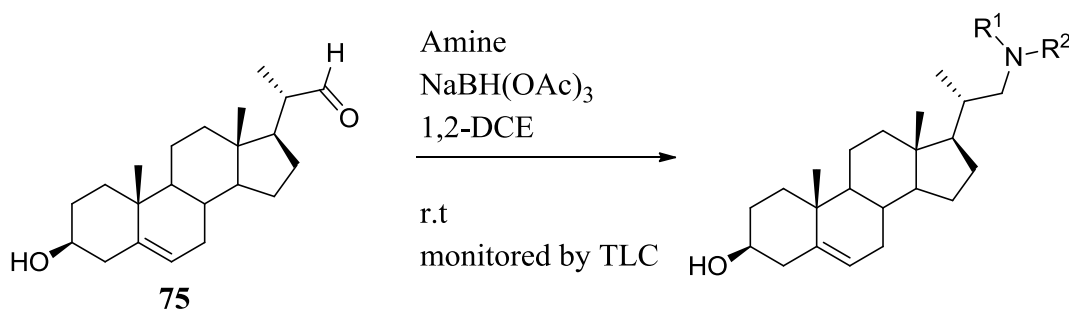


Figure 2.77

2.5.11 Reductive aminations of 3β-hydroxy-20(*S*)-methyl-pregnan-5-ene-21-al 75

Our final objective was to increase our amine derivatives of stigmasterol using 1-(2-hydroxyethyl) piperazine, 4-piperidine methanol and 4-piperidine ethanol, all of which would lead to novel products, Table 2.20.

Table 2.20



Entry	Amine	Product	Compound	Conversion	Isolated Yield
1			91	>95%	24%
2			92	-	^a
3			93	-	^a

a: compounds were not purified due to insufficient quantities of material following the reaction.

As can be seen from Table 2.20, the only successful reaction in this series was entry **1**, the synthesis of **91**. Interestingly, as was the case with the protected analogue **89**, the 1-(2-hydroxyethyl) piperazine derivative did not require purification upon reaction completion. The product **91** is isolated in 24% yield, a comparable result to the protected analogue **89**, which was isolated in 31% yield.

In the ^1H NMR spectrum of this product, a large multiplet between 0.60 and 2.40 ppm obscures many of the characteristic signals corresponding to key changes at the site of reactivity. However, three of the piperazine N-CH_2 signals are seen as a clear multiplet between 2.45 and 2.73 ppm.

Entries **2** and **3** of Table 2.20 show the attempted syntheses of piperidine methanol and piperidine ethanol derivatives **92** and **93** respectively. Unfortunately, both of these products were not successfully isolated. The formation of new multiplets which could be attributed to **92** and **93** are seen in the ^1H NMR spectra for these compounds, however we have no further evidence for the formation of these products.

In the case of **92**, initial diminishment of the aldehyde signal looked promising by ^1H NMR analysis, and the reaction was repeated on the crude material from the first attempt. However,

after repeated attempted reductive aminations, it became clear that complete conversion of the aldehyde would not take place. Impurity peaks were also increasing from the multiple reactions. Purification of the material was not attempted due to the limited amount of product, and the quantity of impurity now present. The reaction was not pursued further at this stage.

It is worth noting that 4-piperidine methanol was the only solid amine we encountered in this project. We believe that the solid nature of the amine may have contributed to the poor reaction outcome in this case, possibly due to decreased solubility in 1,2-dichloroethane.

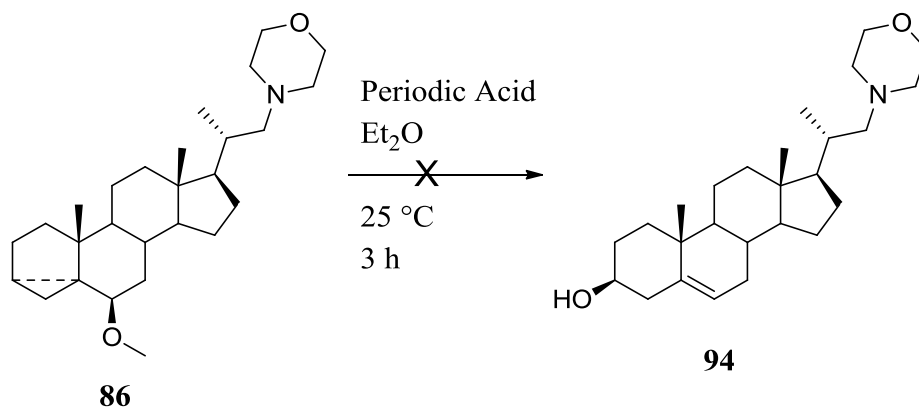
In the case of **93**, though TLC analysis appeared to show complete consumption of the starting material, upon reaction completion and work up, ^1H NMR analysis revealed that there was in fact ~ 70% starting aldehyde remaining. Due to the fact that 0.011 g of crude material was recovered from a starting mass of 0.020 g, and with our knowledge that these reactions yielded in the region of ~30%, the reaction was not repeated.

Due to the difficulty encountered with reductive aminations of **75** with piperidine methanol and piperidine ethanol, further optimisation must be carried out in this area to establish the conditions under which these reactions would be successful.

2.5.12 Deprotection of 20(*S*)-Methyl-6(*R*)-methoxy-3,5-cyclo-21-morpholio-pregnane **86**

As only one fully deprotected stigmastane amine derivative **91** was synthesised in this work, our enthusiasm remained to increase our number of compounds of this nature. Towards the end of this project we resorted to the possibility that deprotection of an amine derivative may be a viable possibility, as our prior methodology was to deprotect the starting aldehyde and subsequently perform the reductive amination.

Periodic acid was employed to deprotect the morpholine derivative **86** in an attempt to synthesise **94** (Scheme 2.69). However, upon reaction completion, the ^1H NMR spectrum showed a complex mixture of unidentifiable materials with no evidence of formation of the desired product.



Scheme 2.69

This result was not an unexpected outcome, as throughout the project the amine moiety has proven to be susceptible to degradation upon further reaction, and indeed under chromatographic conditions. This initial work carried out in this section on stigmasterol derivatives represents the starting point of a vast area of novel chemistry which could be explored in future work. As research into the derivatives of this steroid had not been previously carried out in the group, this initial work provides an excellent platform for further study of these derivatives.

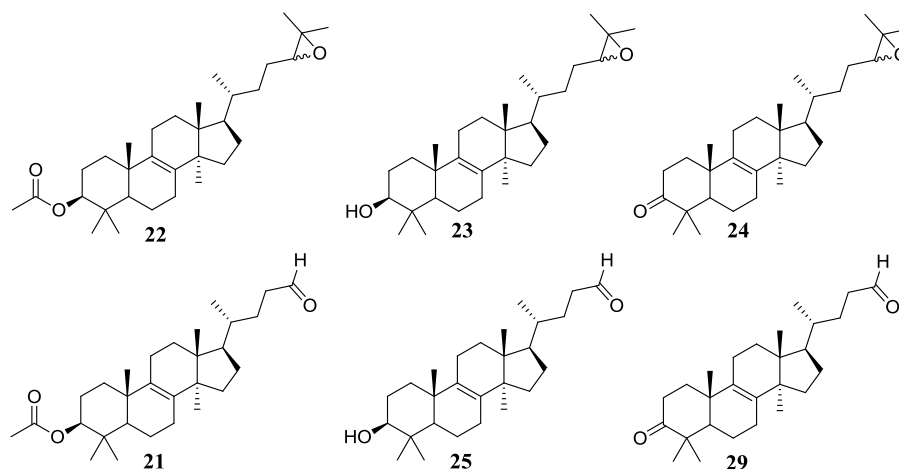
2.6 Conclusions

The combination of the synthesis of novel compounds and the excellent biological activity of a selection of these derivatives leads to the conclusion that this overall project was a great success. The aims of the project were achieved, and previous work was expanded on, particularly utilising non side chain functionalised dihydrolanosterol derivatives and introducing key functionality in terms of oxidation and the useful side chain aldehyde. The main summary points of each section of this project are discussed below, by way of conclusion.

1. Lanosterol Oxidation Products:

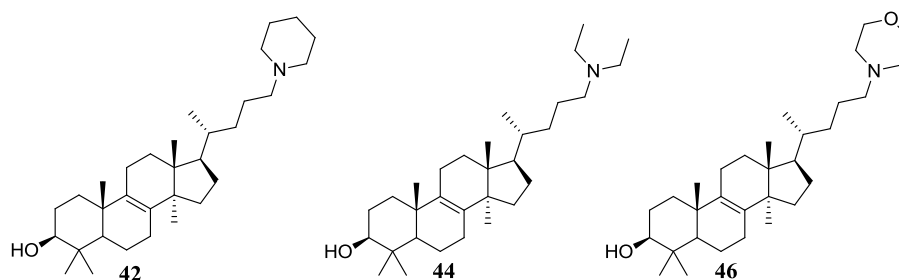
The highlight of this section was the initially promising biological results of the side chain epoxide moieties **22**, **23** and **24**, with the C-3 ketone derivative **24** having an IC₅₀ value of 57 μM in U937 cells and a 16.4 fold increase in apoptotic bodies at 56 μM. These results provided an insight into the structure activity relationships of lanosterol oxidation products, and were used to strategically synthesise target molecules, which showed improved biological activity. Of great significance also was the synthesis of the C-24 aldehyde in the side chain of lanosterol with acetate **21**, alcohol **25** and ketone **29** moieties at C-3. This new series of

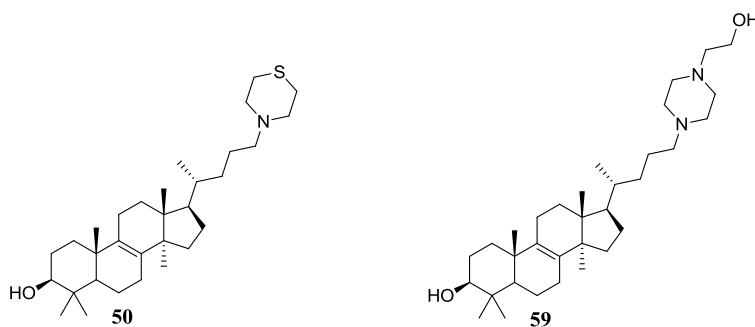
compounds opened up an exciting area of amine chemistry which would ultimately lead to our best biological results to date.



2. Amine derivatives of Lanosterol:

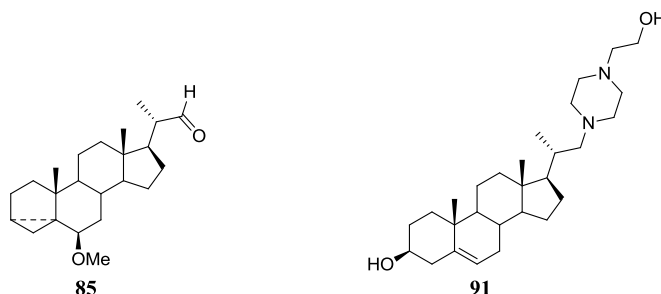
In this section, a synthetic strategy was developed involving reductive amination at the side chain aldehydes. This strategy yielded a panel of novel amine derivatives of lanosterol, incorporating a variety of alkyl amines such as piperidine and diethylamine (compounds **42** and **44** respectively), and heteroatom containing amines including morpholine and thiomorpholine (compounds **46** and **50** respectively). We are very satisfied with this area of the project from a synthetic chemistry point of view, as the successful synthesis and full characterisation of novel derivatives is always challenging. Biological screening of these compounds revealed excellent IC_{50} results for piperidine and 1-(2-hydroxyethyl)piperazine derivatives (compounds **42** and **59**, having IC_{50} values of 1.9 μM and 2.7 μM in U937 cells respectively), comparable to the marketed Etoposide. This biological success was the pinnacle of our project and we were delighted to have achieved such an excellent result. Reductive amination forms the centrepiece of this project, with a large number of novel secondary and tertiary amines derived from both lanosterol and stigmasterol.





3. Cholestane Derivatives of Biologically Active Amines:

The side chain chemistry undertaken on stigmasterol has shown that, despite our best efforts to form the C-22 aldehyde by oxidative cleavage of the C-22,23 epoxide, recourse to ozonolysis was necessary to achieve this transformation. Optimisation of column chromatography work on the C-3 protected C-22 aldehyde **85** led to the isolation of this compound in a sufficiently pure state to carry out reductive amination reactions. Thus four novel amine derivatives were synthesised and fully characterised. One successful synthesis of a C-3 alcohol derivative of stigmasterol with amine side chain **91** gives scope for further work in this area, and we are keen to have this derivative biologically tested. We believe that the initial work carried out on the cholestane derivatives will prove invaluable in future research. Optimisation of key reactions along the synthetic route to amine derivatives will allow for the efficient synthesis of further novel compounds at a later date. Immediate targets in this area would be reaction yield improvement and biological evaluation of the products.



2.7 Future Work

Many areas of this research could potentially be expanded and further investigated. Following on from the synthetic strategies which have been put in place during the course of this work, one could envisage a number of potential target molecules for future synthesis. In the lanosterol oxidation products section, a C-7,8 and C-9,11 diepoxide would be an interesting addition to the novel compounds synthesised in this area. Finally, with much of the necessary methodology work put in place during this project, the stigmasterol chemistry area now shows very promising potential.

2.8 References

1. Yeh, C. T.; Rao, Y. K.; Yao, C. J.; Yeh, C. F.; Li, C. H.; Chuang, S. E.; Luong, J. H. T.; Lai, G. M.; Tzeng, Y. M. *Cancer Lett.* **2009**, 285, 73-79.
2. Male, K. B.; Crowley, S. M.; Collins, S. G.; Tzeng, Y. M.; Luong, J. H. T. *Anal. Methods* **2010**, 2, 870-877.
3. Male, K. B.; Rao, Y. K.; Tzeng, Y. M.; Montes, J.; Kamen, A.; Luong, J. H. T. *Chem. Res. Toxicol.* **2008**, 21, 2127-2133.
4. Luong, J. H. T.; Habibi-Rezaei, M.; Meghrou, J.; Xiao, C.; Male, K. B.; Kamen, A. *Analytical Chemistry* **2001**, 73, 1844-1848.
5. Kampa, M.; Pelekanou, V.; Castanas, E. *Steroids* **2008**, 73, 953-960.
6. Chung, S. K.; Ryoo, C. H.; Yang, H. W.; Shim, J. Y.; Kang, M. G.; Lee, K. W.; Kang, H. I. *Tetrahedron* **1998**, 54, 15899-15914.
7. O'Connell, N. M.; O'Callaghan, Y. C.; O'Brien, N. M.; Maguire, A. R.; McCarthy, F. O. *Tetrahedron* **2012**, 68, 4995-5004.
8. Kavtaradze, L. K.; Manley-Harris, M.; Nicholson, B. K. *Steroids* **2004**, 69, 697-700.
9. Emmons, G. T.; Wilson, W. K.; Schroepfer, G. J. *Magn. Reson. Chem.* **1989**, 27, 1012-1024.
10. Li, T. S.; Li, J. T.; Li, H. Z. *J. Chromatogr. A* **1995**, 715, 372-375.
11. Williams, C. M.; Mander, L. N. *Tetrahedron* **2001**, 57, 425-447.
12. Kavtaradze, L. K.; Manley-Harris, M.; Nicholson, B. K. *Steroids* **2004**, 69, 227-233.
13. Zheng, W. F.; Miao, K. J.; Liu, Y. B.; Zhao, Y. X.; Zhang, M. M.; Pan, S. Y.; Dai, Y. C. *Appl. Microbiol. Biot.* **2010**, 87, 1237-1254.
14. McCarthy, F. O.; Chopra, J.; Ford, A.; Hogan, S. A.; Kerry, J. P.; O'Brien, N. M.; Ryan, E.; Maguire, A. R. *Org. Biomol. Chem.* **2005**, 3, 3059-3065.
15. Zhao, W.; Kong, F. Z. *Bioorgan. Med. Chem.* **2005**, 13, 121-130.
16. Silvestre, S. M.; Salvador, J. A. R. *Tetrahedron* **2007**, 63, 2439-2445.
17. Arsenou, E. S.; Koutsourea, A. I.; Foustieris, M. A.; Nikolaropoulos, S. S. *Steroids* **2003**, 68, 407-414.
18. Shingate, B. B.; Hazra, B. G.; Salunke, D. B.; Pore, V. S. *Tetrahedron Lett.* **2011**, 52, 6007-6010.
19. Zhao, Y. J.; Loh, T. P. *Tetrahedron* **2008**, 64, 4972-4978.
20. Emmons, G. T.; Wilson, W. K.; Schroepfer, G. J. *J. Lipid Res.* **1989**, 30, 133-138.
21. Barton, D. H. R.; Mellows, G.; Widdowso, D. A.; Wright, J. J. *J. Chem. Soc. C-Org.* **1971**, 1142.
22. O'Callaghan, Y.; Kenny, O.; O'Connell, N. M.; Maguire, A. R.; McCarthy, F. O.; O'Brien, N. M. *Biochimie* **2013**, 95, 496-503.
23. Mori, N.; Togo, H. *Tetrahedron* **2005**, 61, 5915-5925.
24. Svennebring, A.; Garg, N.; Nilsson, P.; Hallberg, A.; Larhed, M. *J. Org. Chem.* **2005**, 70, 4720-4725.
25. Parish, E. J.; Sun, H.; Kizito, S.; Boos, T. L. *Molecules* **2000**, 5, 114-117.
26. George, J. H.; McArdle, M.; Baldwin, J. E.; Adlington, R. M. *Tetrahedron* **2010**, 66, 6321-6330.
27. Kennedy, E. M.; P'Pool, S. J.; Jiang, J. H.; Sliva, D.; Minto, R. E. *J. Nat. Prod.* **2011**, 74, 2332-2337.
28. Takatsuto, S.; Watanabe, T.; Gotoh, C.; Kuriyama, H.; Noguchi, T.; Fujioka, S. *J. Chem. Res-S* **1998**, 176-177.
29. Chaudhury, N. K.; Williams, J. G.; Nickolso, R.; Gut, M. *J. Org. Chem.* **1969**, 34, 3759.
30. Arsanious, M. H. N. *Monatsh. Chem.* **1999**, 130, 921-928.
31. Mazzocchi, P. H.; Wilson, P.; Khachik, F.; Klingler, L.; Minamikawa, S. *J. Org. Chem.* **1983**, 48, 2981-2989.
32. Paryzek, Z.; Martynow, J. *Can. J. Chem.* **1988**, 66, 2130-2136.
33. Ruzicka, L.; Montavon, M.; Jeger, O. *Helv. Chim. Acta* **1948**, 31, 818-821.

34. O'Callaghan, Y. C.; Foley, D. A.; O'Connell, N. M.; McCarthy, F. O.; Maguire, A. R.; O'Brien, N. M. *J. Agr. Food. Chem.* **2010**, *58*, 10793-10798.
35. Milner, S. E.; Brunton, N. P.; Jones, P. W.; O'Brien, N. M.; Collins, S. G.; Maguire, A. R. *J. Agr. Food. Chem.* **2011**, *59*, 3454-3484.
36. Herzog, H. L.; Payne, C. C.; Hershberg, E. B. *J. Am. Chem. Soc.* **1955**, *77*, 5324-5327.
37. Lorente, S. O.; Jimenez, C. J.; Gros, L.; Yardley, V.; Luca-Fradley, K.; Croft, S. L.; Urbina, J. A.; Ruiz-Perez, L. M.; Pacanowska, D. G.; Gilbert, I. H. *Bioorgan. Med. Chem.* **2005**, *13*, 5435-5453.
38. Margaretha, P. Reductive Amination of Carbonyl Compounds. 4, pp 405-422. 2011. Science of Synthesis, Knowledge Updates.
39. Hummel, W. Reductive Amination of Keto Acids. 2, pp 1165-1203. 2011. Enzyme Catalysis in Organic Synthesis.
40. AbdelMagid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849-3862.
41. Zhang, C. L.; Tan, C. Y.; Zu, X. Y.; Zhai, X.; Liu, F.; Chu, B. Z.; Ma, X. H.; Chen, Y. Z.; Gong, P.; Jiang, Y. Y. *Eur. J. Med. Chem.* **2011**, *46*, 1404-1414.
42. Pellissier, H. *Tetrahedron* **2004**, *60*, 5123-5162.
43. Wulff, G.; Rohle, G. *Angew. Chem. Int. Edit.* **1974**, *13*, 157-170.
44. Schmidt, R. R. *Angew. Chem. Int. Edit.* **1986**, *25*, 212-235.
45. Whitfield, D. M.; Douglas, S. P. *Glycoconjugate J.* **1996**, *13*, 5-17.
46. Stachulski, A. V.; Jenkins, G. N. *Nat. Prod. Rep.* **1998**, *15*, 173-186.
47. Davis, B. G. *J. Chem. Soc. Perk. T 1* **1999**, 3215-3237.
48. Nakamura, T.; Komori, C.; Lee, Y. Y.; Hashimoto, F.; Yahara, S.; Nohara, T.; Ejima, A. *Biol. Pharm. Bull.* **1996**, *19*, 564-566.
49. Hsu, S. H.; Tsai, T. R.; Lin, C. N.; Yen, M. H.; Kuo, K. W. *Biochem. Bioph. Res. Co.* **1996**, *229*, 1-5.
50. Nohara, T.; Ikeda, T.; Fujiwara, Y.; Matsushita, S.; Noguchi, E.; Yoshimitsu, H.; Ono, M. *J. Nat. Med.* **2007**, *61*, 1-13.
51. Arthan, D.; Svasti, J.; Kittakoo, P.; Pittayakhachonwut, D.; Tanticharoen, M.; Thebtaranonth, Y. *Phytochemistry* **2002**, *59*, 459-463.
52. Ali, Z.; Smillie, T. J.; Khan, I. A. *Carbohydr. Res.* **2013**, *370*, 86-91.
53. Galan, M. C.; Benito-Alifonso, D.; Watt, G. M. *Org. Biomol. Chem.* **2011**, *9*, 3598-3610.
54. Guo, J. A.; Ye, X. S. *Molecules* **2010**, *15*, 7235-7265.
55. Aversa, M. C.; Barattucci, A.; Bonaccorsi, P. *Tetrahedron* **2008**, *64*, 7659-7683.
56. El Ashry, E. S. H.; Awad, L. F.; Atta, A. I. *Tetrahedron* **2006**, *62*, 2943-2998.
57. Lee, Y. S.; Rho, E. S.; Min, Y. K.; Kim, B. T.; Kim, K. H. *J. Carbohydr. Chem.* **2001**, *20*, 503-506.
58. Davis, B. G.; Wood, S. D.; Maughan, M. A. T. *Can. J. Chem.* **2002**, *80*, 555-558.
59. Rodriguez-Perez, T.; Lavandera, I.; Fernandez, S.; Sanghvi, Y. S.; Ferrero, M.; Gotor, V. *Eur. J. Org. Chem.* **2007**, 2769-2778.
60. Schneide, J. J.; Bhacca, N. S. *J. Org. Chem.* **1969**, *34*, 1990-&.
61. Davis, B. G.; Lloyd, R. C.; Jones, J. B. *J. Org. Chem.* **1998**, *63*, 9614-9615.
62. Weihrauch, J. L.; Gardner, J. M. *J. Am. Diet. Assoc.* **1978**, *73*, 39-47.
63. Foley, D. A.; O'Callaghan, Y.; O'Brien, N. M.; McCarthy, F. O.; Maguire, A. R. *J. Agr. Food. Chem.* **2010**, *58*, 1165-1173.
64. Spencer, T. A.; Li, D. S.; Russel, J. S.; Tomkinson, N. C. O.; Willson, T. M. *J. Org. Chem.* **2000**, *65*, 1919-1923.
65. Marino, J. P.; deDios, A.; Anna, L. J.; delaPradilla, R. F. *J. Org. Chem.* **1996**, *61*, 109-117.
66. Hazra, B. G.; Joshi, P. L.; Bahule, B. B.; Argade, N. P.; Pore, V. S.; Chordia, M. D. *Tetrahedron* **1994**, *50*, 2523-2532.
67. Hang, J. L.; Dussault, P. *Steroids* **2010**, *75*, 879-883.
68. Brown, H. C.; Narasimhan, S.; Choi, Y. M. *Synthesis-Stuttgart* **1981**, 441-442.
69. Brown, H. C.; Choi, Y. M.; Narasimhan, S. *J. Org. Chem.* **1982**, *47*, 3153-3163.
70. Samano, V.; Robins, M. J. *J. Org. Chem.* **1991**, *56*, 7108-7113.

71. Evans, D. A.; Ellman, J. A.; Dorow, R. L. *Tetrahedron Lett.* **1987**, 28, 1123-1126.
72. Nes, W. D. *Bba-mol. Cell. Biol. L.* **2000**, 1529, 63-88.

Chapter Three

Experimental



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3.1 General Procedures

Solvents were distilled prior to use as follows: dichloromethane was distilled from phosphorous pentoxide, ethyl acetate was distilled from potassium carbonate, hexane was stored and distilled prior to use. Organic phases were dried using anhydrous magnesium sulfate. All commercial reagents were used without further purification.

Proton (300 MHz and 400 MHz) NMR and carbon (75.5 MHz) NMR spectra were recorded on Bruker Avance 300 NMR and 400 NMR spectrometers. All spectra were recorded at 20 °C in deuterated chloroform (CDCl₃) unless otherwise stated, using tetramethylsilane (TMS) as internal standard. Chemical shifts, δ_{H} and δ_{C} , are expressed as parts per million (ppm).

Splitting patterns in ¹H NMR spectra are designated as s (singlet), d (doublet), t (triplet) and m (multiplet). Coupling constants (*J*) are quoted in Hz.

¹³C NMR spectra were assigned with the aid of DEPT experiments run in the DEPT-90, DEPT-135 and DEPT-Q modes.

Infrared spectra were measured as potassium bromide discs for solids, or thin films on sodium chloride plates for oils, on a Perkin-Elmer Paragon 1000 FT-IR spectrometer in the range 4000 to 450 cm⁻¹.

Thin layer chromatography (TLC) was carried out on pre-coated silica gel plates (Merck Silica Gel 60, F₂₅₄). Wet flash chromatography was performed using Merck silica gel 60 and the fractions are reported in the order with which they eluted unless otherwise stated. Visualisation of compounds on TLC plates was achieved by ultra-violet light (254 nm) detection or vanillin staining.

Melting points were carried out on a uni-melt Thomas Hoover Capillary melting point apparatus and are uncorrected.

Elemental analyses were performed by the Microanalysis Laboratory, National University of Ireland, Cork, using Perkin-Elmer 240 and Exeter Analytical CE440 elemental analysers.

Low resolution mass spectra were recorded on a Waters Quattro Micro triple quadrupole spectrometer on electrospray ionisation (ESI) mode using 50% water/acetonitrile containing

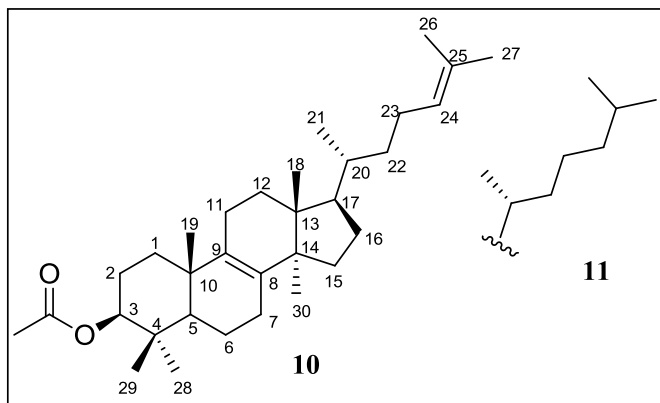
Chapter 3: Experimental

0.1% formic acid as eluent. Samples were submitted for analysis dissolved in 1 mL of acetonitrile, unless otherwise stated.

High resolution mass spectra were recorded on a Waters LCT Premier Time of Flight spectrometer in electrospray ionisation mode, eluent and sample solvent being as described above.

3.2 Lanosterol Oxidation Products

3 β -Acetoxy-5 α -lanosta-8,24-diene **10** and 3 β -acetoxy-5 α -lanost-8-ene¹ **11**.



Acetic anhydride (0.344 g, 3.37 mM) was added to a stirring solution of a 50:50 mixture of lanosterol **6** and dihydrolanosterol **7** (1.000 g, 2.34 mmol) dimethylaminopyridine (0.020 g, 0.07 mM) and pyridine (1.52 mL, 18.79 mM) in dichloromethane (10 mL). The solution was stirred under a

blanket of nitrogen at room temperature for 24 h. The solution was washed sequentially with 5% hydrochloric acid (3 \times 10 mL), 10% sodium hydrogen carbonate (3 \times 10 mL), brine (1 \times 10 mL) and water (1 \times 10 mL) respectively. The organic layer was dried over MgSO₄ and concentrated *in vacuo* to give a pale yellow residue. The residue was recrystallised from acetone to yield an inseparable mixture of 3 β -acetoxy-5 α -lanosta-8,24-diene **10** and 3 β -acetoxy-5 α -lanost-8-ene **11** (50:50 ratio) as a white solid (0.353 g, 32%): mp 110 – 118 °C (literature,¹ 125 – 129 °C); ν_{\max} (KBr)/cm⁻¹: 2953, 1736. Exact mass calculated for C₃₂H₅₃O₂ [M + H]⁺ 469.4046, found 469.4046; Exact mass calculated for C₃₂H₅₅O₂ [M + H]⁺ 471.4202, found 471.4193.

3 β -Acetoxy-5 α -lanosta-8,24-diene^{1,2} **10**

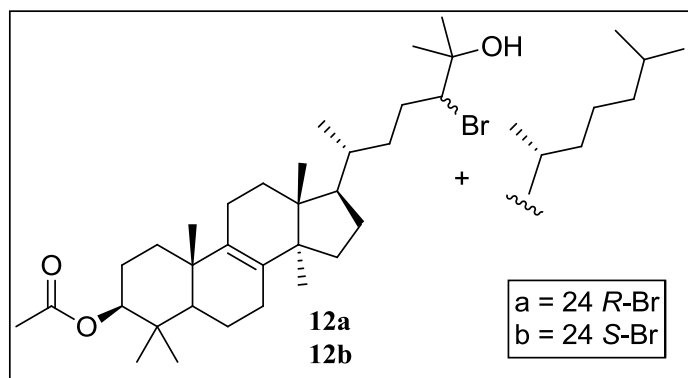
δ_{H} (300 MHz, CDCl₃) 0.69 – 1.76 [51H, m, containing: 0.69 (3H, s, 18-CH₃), 0.85 – 0.92 (12H, m, 4 \times CH₃: 30-CH₃, 29-CH₃, 28-CH₃, 21-CH₃), 1.00 (3H, s, 19-CH₃), 1.12 – 1.21 (1H, m, 5-CH), 1.25-1.37 (1H, m, 20-CH), 1.60 (3H, s, 27-CH₃), 1.68 (3H, s, 26-CH₃), 2.05 (3H, s, Ac-CH₃)] 4.50 (1H, dd, *J* 11.2, 4.7, 3-CH), 5.10 (1H, t, *J* 7.0, 24-CH); δ_{C} (75 MHz, CDCl₃) 15.8 (18-CH₃), 16.5 (29-CH₃), 17.7 (27-CH₃), 18.1 (6-CH₂), 18.6 (21-CH₃), 19.2 (19-CH₃), 21.0 (11-CH₂), 21.4 (OAc, CH₃), 24.2 (2-CH₂), 24.3 (30-CH₃), 24.9 (23-CH₂), 25.8 (26-CH₃), 26.4 (7-CH₂), 27.9 (28-CH₃), 28.2 (16-CH₂), 30.8 (15-CH₂), 31.0 (12-CH₂), 35.3 (1-CH₂), 36.4 (20-CH), 36.5 (22-CH₂), 36.9 (10-quaternary C), 37.8 (4-quaternary C), 44.5 (13-quaternary C), 49.8 (14-quaternary C), 50.4 (5-CH or 17-CH), 50.5 (5-CH or 17-CH),

80.9 (3-CH), 125.3 (24-CH), 130.9 (25-quaternary C), 134.2 (9-quaternary C), 134.5 (8-quaternary C), 171.0 (quaternary C, C=O).

3 β -Acetoxy-5 α -lanost-8-ene^{1,2} **11**

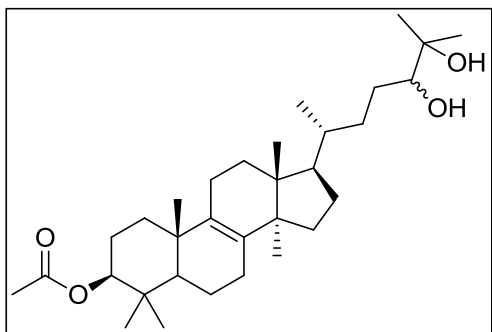
δ_H (300 MHz, CDCl₃) 0.69 – 1.76 [54H, m, containing: 0.69 (3H, s, 18-CH₃), 0.85 – 0.92 (12H, m, 4 \times CH₃: 30-CH₃, 29-CH₃, 28-CH₃, 21-CH₃), 1.00 (3H, s, 19-CH₃), 1.12 – 1.21 (1H, m, 5-CH), 1.25 – 1.37 (1H, m, 20-CH), 1.60 (3H, s, 27-CH₃), 1.68 (3H, s, 26-CH₃), 2.05 (3H, s, Ac-CH₃)] 4.50 (1H, dd, J 11.2, 4.7, 3-CH), ¹³C NMR spectrum contains a majority of overlapping signals with key differences at C-24, C-25, C-26 and C-27. δ_C (75 MHz, CDCl₃) 15.7 (18-CH₃), 16.5 (29-CH₃), 18.1 (6-CH₂), 18.7 (21-CH₃), 19.2 (19-CH₃), 21.0 (11-CH₂), 21.4 (OAc, CH₃), 22.6 (26-CH₃), 22.9 (27-CH₃), 24.1 (23-CH₂), 24.2 (2-CH₂), 24.3 (30-CH₃), 26.4 (7-CH₂), 27.9 (28-CH₃), 28.0 (25-CH), 28.2 (16-CH₂), 30.4 (15-CH₂), 31.0 (12-CH₂), 35.3 (1-CH₂), 36.3 (20-CH), 36.5 (22-CH₂), 36.9 (10-quaternary C), 37.8 (4-quaternary C), 39.5 (24-CH₂), 44.5 (13-quaternary C), 49.8 (14-quaternary C), 50.4 (5-CH or 17-CH), 50.5 (5-CH or 17-CH), 80.9 (3-CH), 134.2 (9-quaternary C), 134.5 (8-quaternary C), 171.0 (quaternary C, C=O).

24(*R,S*)-3 β -Acetoxy-24-bromo-25-hydroxy-5 α -lanost-8-ene¹ **12** and 3 β -acetoxy-5 α -lanost-8-ene **11**



N-bromosuccinimide (1.350 g, 7.59 mM) was added to a stirring solution of the inseparable 50:50 mixture of lanosterol acetate **10** and dihydrolanosterol acetate **11** (4.449 g, 9.49 mM) in acetone (450 mL), deionised water (7 mL) and hypophosphorous acid (1.19 mL,

21.80 mM). The solution was stirred at room temperature for 5 min, whereupon NaHCO₃ (1.350 g, 16.13 mM) was added. The solution was concentrated *in vacuo* to yield a mixture of 24(*R,S*)-3 β -acetoxy-24-bromo-25-hydroxy-5 α -lanost-8-ene **12** and 3 β -acetoxy-5 α -lanost-8-ene **11** (7.960 g) which was carried forward without purification.

24(*R,S*)-3 β -Acetoxy-24,25-dihydroxy-5 α -lanost-8-ene¹ 13a and 13b

A mixture of 24(*R,S*)-3 β -acetoxy-24-bromo-25-hydroxy-5 α -lanost-8-ene **12** and 3 β -acetoxy-5 α -lanost-8-ene **11** (6.56 g, 11.6 mM) was dissolved in 2-propanol (196 mL), deionised water (65 mL), and hypophosphorous acid (4.09 mL, 74.8 mM). NaHCO₃ (6.045 g, 71.88 mM) was added and the mixture was refluxed at 160 °C for 4 h. After this

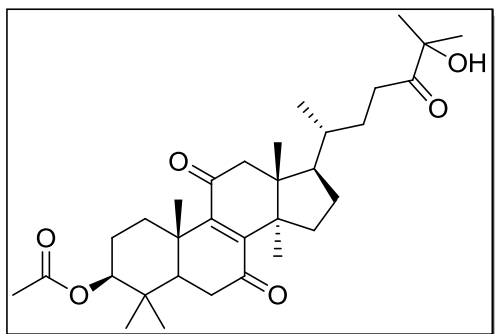
time period, water (200 mL) was added to the reaction mixture. The resulting solid was filtered through a sintered Büchner funnel. The solid was washed with water and tested with pH paper, while in a wet state, until pH 7 was obtained. The crude product was purified by column chromatography using dichloromethane. The first fraction from the column was 3 β -acetoxy-5 α -lanost-8-ene **11**, isolated as a white solid (2.06 g, 41%). The second fraction from the column was a 1:1 diastereomeric mixture of 24(*R,S*)-3 β -acetoxy-24,25-dihydroxy-5 α -lanost-8-ene **13a and 13b** isolated as a white solid (2.59 g, 72%); mp 121 – 125 °C (literature,¹ 172 – 175 °C); ν_{max} (KBr)/cm⁻¹: 3431, 2966, 1732;

3 β -Acetoxy-24(*R*),25-dihydroxy-5 α -lanost-8-ene^{1,2} 13a

δ_{H} (300 MHz, CDCl₃) 0.69 (3H, s, 18-CH₃), 0.84 – 0.94 (12H, m, 28-CH₃, 29-CH₃, 30-CH₃, 21-CH₃), 1.00 (3H, s, 19-CH₃), 1.09 – 1.16 (1H, m, 5-CH), 1.21 (3H, s, 27-CH₃), 1.25 – 1.75 (5H, m, 26-CH₃, 20-CH, 17-CH), 2.05 (3H, s, OAc, CH₃), 3.29 (1H, d, *J* 9.5, 24(*R*)-CH), 4.50 (1H, dd, *J* 11.2, 4.7, 3-CH). δ_{C} (75 MHz, CDCl₃) 15.8 (18-CH₃), 16.5 (29-CH₃), 18.0 (6-CH₂), 18.5 (21-CH₃), 21.0 (11-CH₂), 21.3 (OAc, CH₃), 23.2 (26-CH₃), 26.4 (7-CH₂), 27.9 (2 of β or 23 of α), 28.2 (28-CH₃), 30.8 (15-CH₂), 31.0 (12-CH₂), 33.6 (22-CH₂), 35.2 (1-CH₂), 36.9 (10-quaternary C), 36.7 (20-CH), 37.8 (4-quaternary C), 44.5 (13-quaternary C), 49.8 (14quaternary C), 50.5 (5-CH), 53.4 (quaternary C), 63.4 (CH), 73.2 (25-quaternary C), 78.7 (24(*R*)-CH), 81.0 (3-CH), 134.2 (9-quaternary C), 134.4 (8-quaternary C), 171.1 (quaternary C, C=O).

3 β -Acetoxy-24(*S*),25-dihydroxy-5 α -lanost-8-ene^{1,2} 13b

δ_H (300 MHz, $CDCl_3$) 0.69 (3H, s, 18- CH_3), 0.84 – 0.94 (12H, m, 28- CH_3 , 29- CH_3 , 30- CH_3 , 21- CH_3), 1.00 (3H, s, 19- CH_3), 1.09 – 1.16 (1H, m, 5-CH), 1.21 (3H, s, 27- CH_3), 1.25 – 1.75 (5H, m, 26- CH_3 , 20-CH, 17-CH), 2.05 (3H, s, OAc, CH_3), 3.34 (1H, s, 24(*S*)-CH), 4.50 (1H, dd, J 11.2, 4.7, 3-CH); δ_C (75 MHz, $CDCl_3$) 15.8 (18- CH_3), 16.5 (29- CH_3), 18.0 (6- CH_2), 18.8 (21- CH_3), 21.0 (11- CH_2), 21.3 (OAc, CH_3), 23.2 (26- CH_3), 26.4 (7- CH_2), 27.9 (2- CH_2 of β or 23- CH_2 of α), 28.2 (28- CH_3), 30.8 (15- CH_2), 31.0 (12- CH_2), 33.2 (22- CH_2), 35.2 (1- CH_2), 36.9 (10-quaternary C), 36.7 (20-CH), 37.8 (4-quaternary C), 44.5 (13-quaternary C), 49.8 (14-quaternary C), 50.5 (5-CH), 53.4 (quaternary C), 63.4 (CH), 73.2 (25-quaternary C), 79.6 (24(*S*)-CH), 81.0 (3-CH), 134.2 (9-quaternary C), 134.4 (8-quaternary C), 171.1 (quaternary C, C=O).

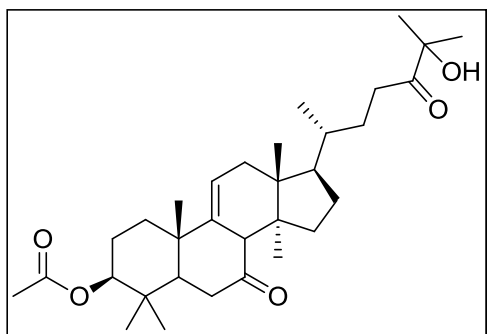
3 β -Acetoxy-5 α -lanost-8-ene-7,11,24-one-25-ol 14

A solution of chromium trioxide (0.955 g, 9.55 mM) in acetic acid (30 mL, 90% v/v) heated to 80 °C was added to a stirring solution of 1:1 24(*R,S*) 3 β -acetoxy-24,25-dihydroxy-5 α -lanost-8-ene **13a** and **13b** (1.200 g, 2.38 mM) in glacial acetic acid (48 mL) also heated to 80 °C³. The solution was stirred for 50 min at 80 °C, removed from the oil

bath and allowed to cool to room temperature. The reaction was then cooled on an ice bath for 1 h, poured into water (100 mL) and extracted with chloroform (3 \times 50 mL). The combined organic extracts were washed with water (4 \times 25 mL) dried over $MgSO_4$ and concentrated under reduced pressure to give the crude product consisting of 3 β -acetoxy-5 α -lanost-8-ene-7,11,24-one-25-ol **14** and 3 β -acetoxy-5 α -lanost-9(11)-en-7,24-one-25-ol **15** as a yellow solidifying oil. Purification by column chromatography (hexane:ethyl acetate 80:20) led to the isolation of the desired product, as the second fraction from the column, 3 β -acetoxy-5 α -lanost-8-ene-7,11,24-one-25-ol **14** as a yellow solid (0.392 g, 31%); mp 186 – 190 °C; Found C, 73.18; H, 9.41. $C_{32}H_{48}O_6$ requires C, 72.69; H 9.15%; ν_{max} (KBr)/ cm^{-1} : 3367, 1734, 1676; δ_H (300 MHz, $CDCl_3$) 0.80 (3H, s, 18- CH_3), 0.90 (3H, s, 26- CH_3), 0.91 – 1.90 [20H, m, containing 0.96 (3H, s, 19- CH_3), 0.90 – 1.27 (9H, m, 21- CH_3 , 30- CH_3 , 28- CH_3

or 29-CH₃), 1.17 (3H, s, 28-CH₃ or 29-CH₃), 1.33 (3H, s, 27-CH₃), 2.00 – 2.50 [5H, m, containing 2.06 (3H, s, OAc CH₃)], 2.60 (1H, d, *J* 15.8 12β-CH), 2.78 (1H, d, *J* 16.1, 12α-CH), 2.89 (1H, dt, *J* 13.6, 3.5, 1β-CH), 4.55 (1H, dd, *J* 5.4, 11.0, 3-CH); δ_C (75 MHz, CDCl₃) 16.5 (29-CH₃), 16.8 (18-CH₃), 17.6 (27-CH₃), 18.1 (21-CH₃), 21.3 (OAc, CH₃ or 30-CH₃), 23.9 (2-CH₂), 25.9 (28-CH₃), 27.2 (16-CH₂), 27.8 (19-CH₃), 30.7 (22-CH₂), 31.1 (23-CH₂), 32.1 (15-CH₂), 33.7 (1-CH₂), 35.7 (20-CH), 36.2 (6-CH₂), 37.7 (10-quaternary C), 39.6 (4-quaternary C), 47.5 (13-quaternary C), 48.9 (5-CH), 49.0 (14-quaternary C), 50.1 (17-CH), 51.5 (12-CH₂), 79.3 (3-CH), 150.5 (8-quaternary C), 151.7 (9-quaternary C), 170.9 (quaternary C, OAc C=O), 179.6 (quaternary C, C=O), 201.9 (C=O, 11-quaternary C), 202.2 (C=O, 7-quaternary C); *m/z* (ESI⁺) 528 [M]⁺ (14%), 473 (100); Exact mass calculated for C₃₂H₄₉O₆ [M + H]⁺ 529.3529, found 529.3542.

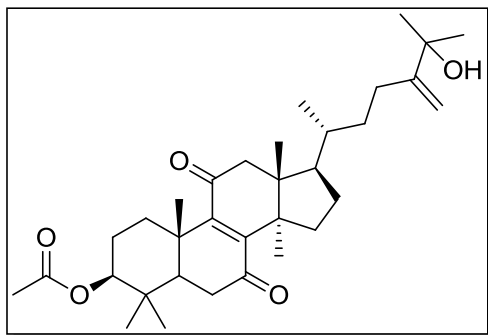
3β-Acetoxy-5α-lanost-9(11)-en-7,24-one-25-ol **15**



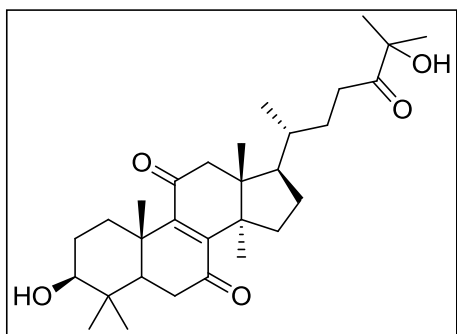
The first fraction from the column described above was isolated as a yellow oil containing a mixture of numerous products (0.088 g), one of which being tentatively assigned as 3β-acetoxy-5α-lanost-9(11)-en-7,24-one-25-ol **15**.

Characteristic signals, within the mixture of numerous products, for this tentative assignment are described below:

δ_H (300 MHz, CDCl₃) 0.68 (3H, s, 18-CH₃), 0.76 (3H, s, 30-CH₃), 0.83 (3H, s, 28-CH₃), 0.85–0.92 (9H, m, 21-CH₃, 26-CH₃, 27-CH₃), 0.93 (3H, s, 29-CH₃), 1.12 (3H, s, 19-CH₃), 1.27–1.98 (3H, m, 5-CH, 17-CH, 20-CH), 2.07 (3H, s, OAc, CH₃), 2.89 (1H, s, H-8β), 4.52 (1H, dd, *J* 10.8, 4.3, H-3), 5.40–5.41 (1H, m, H-11); ν_{max} (film)/cm⁻¹: 2967, 1734, 1708.

Attempted Synthesis of 3 β -Acetoxy-5 α -lanost-8-ene-7,11-one-24-ene-25-ol **33**

Sodium hydride (0.006 g, 0.18 mM) was placed in a dry 100 mL three neck round bottom flask. Dimethylsulfoxide (0.7 mL) was added immediately and the solution was heated to 75 °C for 1 h. The methylsulfinyl carbanion solution was cooled to 18 °C and methyltriphenylphosphonium bromide (0.068 g, 0.19mM) in dimethylsulfoxide (0.2 mL) was added dropwise. After addition was complete the solution was allowed to warm to room temperature over a 30 min period. Ketone **14** (0.080 g, 0.15 mM) in dimethylsulfoxide (10 mL) was added dropwise to the Wittig reagent and the reaction was allowed to stir at room temperature for 6 h.⁴ After this time, cold water (50 mL) was added to the reaction solution. The product was extracted with hexane (4 × 40 mL). The hexane remained clear and the orange aqueous layer was not observed to change. The combined hexane extracts were washed with water (2 × 40 mL) and dried over magnesium sulfate. The solvent was removed under reduced pressure and ¹H NMR analysis was obtained. The ¹H NMR spectrum showed no evidence of formation of the desired product **33**, and also no evidence of any steroidal material in this sample. The aqueous layer was extracted with dichloromethane (3 × 40 mL) and the orange colour of the aqueous layer was observed to transfer to the organic layer. The combined dichloromethane extracts were washed with water (2 × 20 mL), dried over magnesium sulfate and concentrated under reduced pressure to yield an orange oil. Analysis of the ¹H NMR spectrum of the crude product showed no evidence of the formation of the desired product, and also no evidence of any steroidal material in this sample. The reaction was not pursued further.

5 α -Lanost-8-ene-7,11,24-one-3,25-ol **16****Method A:**

10% KOH in EtOH (10 mL) was added to 3 β -acetoxy-5 α -lanost-8-ene-7,11,24-one-25-ol **14** (0.075 g, 0.14 mM) and the solution was heated to 60 °C for 2 h. The resulting mixture was poured onto ice water and

allowed to stand for 6 h. 10% HCl (2 mL) was added and the precipitate was isolated by suction filtration¹ which yielded the desired product **16** as a yellow solid (0.048 g, 70%); mp 181 – 183 °C ν_{\max} (KBr)/cm⁻¹: 3400, 2918, 1670; δ_{H} (300 MHz, CDCl₃) 0.80 (3H, s, 18-CH₃), 0.89 (3H, s, 26-CH₃), 0.90 – 1.27 (9H, m, 21-CH₃, 30-CH₃, 28-CH₃ or 29-CH₃), 1.02 (3H, s, 19-CH₃), 1.18 (3H, s, 28-CH₃ or 29-CH₃), 1.30 (3H, s, 27-CH₃), 1.31 – 2.52 (20H, m), 2.60 (1H, d, *J* 15.8 12 β -CH), 2.78 (1H, d, *J* 16.1, 12 α -CH), 2.89 (1H, dt, *J* 13.6, 3.5, 1 β -CH), 3.23 (1H, dd, *J* 11.0, 5.4, 3-CH); δ_{C} (75 MHz, CDCl₃) 15.5 (CH₃), 16.8 (29-CH₃), 17.5 (18-CH₃), 18.1 (27-CH₃), 25.8 (26-CH₃), 27.2 (CH₂), 27.5 (16-CH₂), 27.8 (28-CH₃), 29.7 (15-CH₂), 30.7 (22-CH₂), 32.1 (23-CH₂), 34.0 (1-CH₂), 35.7 (20-CH), 36.4 (2-CH₂), 38.8 (10-quaternary C), 39.7 (4-quaternary C), 47.3 (13-quaternary C), 48.7 (5-CH), 48.9 (14-quaternary C), 50.1 (17-CH), 51.5 (12-CH₂), 77.6 (3-CH), 150.5 (8-quaternary C), 151.8 (9-quaternary C) 177.8 (quaternary C, C=O), 202.2 (11-quaternary C, C=O), 202.3 (7-quaternary C, C=O); exact mass calculated for C₃₀H₄₆O₅ [M - H]⁺ 485.3267, found 485.3982.

Method B⁵:

Potassium carbonate (0.025 g, 0.19 mM) was dissolved in water (10 mL) and added to a suspension of 3 β -acetoxy-5 α -lanost-8-ene-7,11,24-one-25-ol **14** (0.090 g, 0.17 mM) in methanol stirring at room temperature for 5 min. The reaction mixture was stirred at room temperature for 24 h. The mixture was partitioned between water and ethyl acetate, washed with water (2 \times 50 mL) and brine (2 \times 50mL), dried over MgSO₄ and concentrated under reduced pressure to yield starting material **14** and unidentified materials by ¹H NMR analysis (0.090 g).

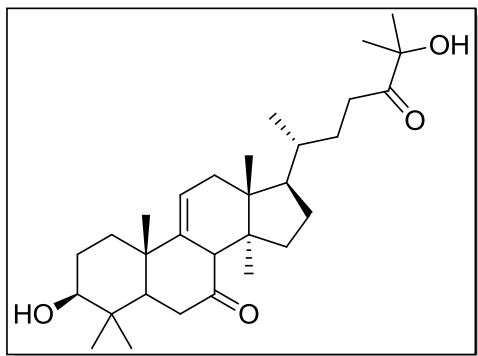
Method C:

Procedure as outlined in Method B, using the substrate isolated from method B and twice the equivalents of potassium carbonate (0.047 g, 0.34 mM). Again, starting material 3 β -acetoxy-5 α -lanost-8-ene-7,11,24-one-25-ol **14** was isolated (0.81g, 90% recovery). Trace amounts of unidentified materials were also present by ¹H NMR analysis.

Method D:

Acetyl chloride (0.05 mL, 0.77 mM) was added dropwise to a solution of the material isolated from method C (0.081 g, 0.15 mM)* dissolved in a 50:50 mixture of anhydrous dichloromethane and anhydrous methanol at 0 °C. The solution was then stirred for 72 h at room temperature after which time the reaction mixture was neutralised with triethylamine (0.11 mL 0.77 mM) and poured into water (100 mL). The solution was extracted with dichloromethane (3 × 30 mL), washed with water (4 × 25 mL), washed with brine (2 × 25mL), dried over MgSO₄ and concentrated under reduced pressure to yield 3β-acetoxy-5α-lanost-8-ene-7,11,24-one-25-ol **14** : 5α-lanost-8-ene-7,11,24-one-25,3-ol **16** (1 : 1) (0.080 g).

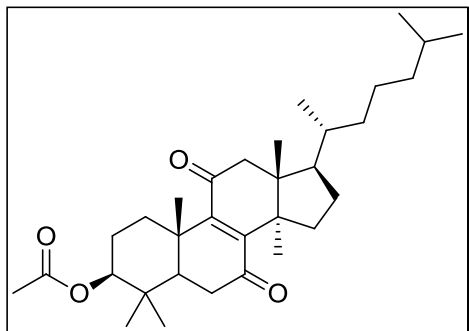
*Starting material was contaminated from previous attempts of the reaction.

5α-Lanost-9(11)-ene-7,24-dione-3β,25-diol **17**

10% KOH in ethanol (4.5 ml) was added to 3β-acetoxy-5α-lanost-9(11)-en-7,24-one-25-ol **15** (0.146 g) and the solution was heated to 60 °C for 2 h. The resulting mixture was poured onto ice water left to stand for 6 h. The mixture was acidified to pH 2 with dropwise addition of 10% HCl.¹ Suction filtration yielded 5α-lanost-9(11)-ene-7,24-dione-3β,25-diol

17, among other materials, as a white solid (0.02 g, 15%). Mp 198 – 200 °C; ν_{max} (film)/cm⁻¹: 3410, 1707, 1673.

¹H NMR analysis of the crude material revealed a complex mixture of products. A shift in the 3-CH peak from 4.52 ppm to 3.30 ppm indicated successful alcohol deprotection. However other materials in the ¹H NMR spectrum of the crude material were unidentifiable due to possibly degraded starting material.

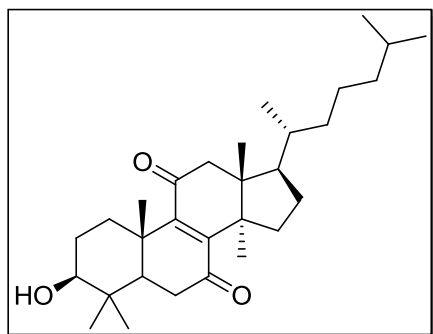
3 β -Acetoxy-5 α -lanost-8-ene-7,11-dione³ **18**

A solution of chromium trioxide (0.709 g, 7.10 mM, 4 eq) in acetic acid (30 mL, 90% v/v) heated to 80 °C was added to a stirring solution of 3 β -acetoxy-5 α -lanost-8-ene **11** (0.835 g, 1.77 mM) in glacial acetic acid (40 mL) also heated to 80 °C. The solution was stirred for 1 h at 80 °C,³ removed from the oil bath and allowed to cool to room temperature. The reaction

was then cooled on an ice bath for 1 h and poured on to water (100 mL). The solution was extracted with chloroform (3 \times 30 mL) and the combined organic extracts were washed with water (2 \times 30 mL), dried over MgSO₄ and concentrated under reduced pressure to give the crude product as a green/yellow solidifying oil (0.740 g). ¹H NMR analysis of the crude material showed the reaction had gone to completion, with minor impurity peaks being observed at 4.02 ppm and 4.20 ppm. Purification by repeated column chromatography using 70:30 hexane : ethyl acetate removed the impurity peak at 4.05 ppm and led to the isolation of the desired product 3 β -acetoxy-5 α -lanost-8-ene-7,11-dione **18** as a yellow solid (0.265 g 30%), mp 152 – 154 °C (literature,⁶ 159 – 161 °C); ν_{max} (KBr)/cm⁻¹: 1737, 1716, 1676. δ_{H} (300 MHz, CDCl₃) 0.79 (3H, s, 18-CH₃), 0.85 – 0.90 (12H, m, containing 4 \times CH₃, 19-CH₃, 21-CH₃, 26-CH₃, 27-CH₃), 0.95 (3H, s, 29-CH₃ or 28-CH₃), 1.17 (3H, s, 29-CH₃ or 28-CH₃), 1.32 (3H, s, 30-CH₃) 1.49 – 1.80 (7H, m, 20-CH, 16-CH₂, 15-CH₂, 17-CH, 25-CH), 1.92 – 2.05 (1H, m), 2.06 (3H, s, OAc, CH₃), 2.10 – 2.20 (1H, m), 2.40 – 2.55 (2H, m), 2.62 (1H, d, *J* 15.9, 12 β -CH), 2.77 (1H, d, *J* 15.9, 12 α -CH), 2.90 (1H, dt, *J* 13.7, 3.6, 1 β -CH), 4.53 (1H, dd, *J* 11.0, 5.5, 3-CH); δ_{C} (75 MHz, CDCl₃) 16.5 (29-CH₃), 16.8 (CH₃), 17.5 (18-CH₃), 18.6 (CH₃), 21.3 (30-CH₃), 22.5 (26-CH₃), 22.8 (27-CH₃), 23.9 and 24.0 (23-CH₂ or 2-CH₂), 25.9 (28-CH₃), 27.3 (16-CH₂), 27.81 (OAc, CH₃ or 19-CH₃), 27.98 (25-CH), 32.2 (15-CH₂), 33.8 (1-CH₂), 36.2 (20), 36.20 (6-CH₂), 36.3 (22-CH₂), 37.7 (4), 39.4 (24-CH₂), 39.6 (10-quaternary C), 47.5 (13-quaternary C), 49.0 (14-quaternary C), 49.1 (17-CH), 50.1 (5-CH), 51.6 (12-CH₂), 79.3 (3-CH), 150.7 (8-quaternary C), 151.7 (9-quaternary C), 170.8 (OAc), 202.0 (11-quaternary C, C=O), 202.5 (7-quaternary C, C=O); *m/z* 499 [M + H]⁺ (28%), 267 (100); exact mass calculated for C₃₂H₅₁O₄ [M + H]⁺ 499.3787, found 499.3795.

Method B⁶:

70% ¹Butyl hydroperoxide (0.95 mL, 9.90 mM) was added dropwise to a stirring solution of an inseparable mixture of lanosterol acetate **10** and dihydrolanosterol acetate **11** (1:1) (0.150 g, 0.32 mM) and ruthenium trichloride (0.0009 g, 0.004 mM) in hexane (20 mL). The reaction solution was stirred at room temperature for 6 h. Saturated aqueous sodium bicarbonate (20 mL) was added to the reaction solution. The solution was then washed with sodium thiosulfate (2 × 20 mL) and brine (20 mL). The organic layer was dried over magnesium sulfate and concentrated under reduced pressure to yield the crude product (0.136 g). Analysis of the ¹H NMR spectrum of the crude material showed only starting materials present, with no evidence of formation of the desired product. Likewise, no product formation was observed upon analysis of the carbonyl region of the ¹³C NMR spectrum.

5 α -lanost-8-ene-7,11 dione 3-ol **19**

10% KOH in ethanol (6 mL) was added to 3 β -acetoxy-5 α -lanost-8-ene-7,11 dione **18** (0.265 g, 0.53 mM) and the solution was heated to 60 °C for 2 h. The resulting mixture was poured onto ice water allowed to stand for 6 h.¹ Suction filtration yielded the crude product as an off white solid (0.237 g). Analysis of the ¹H NMR spectrum of the crude material showed the reaction had gone 92%

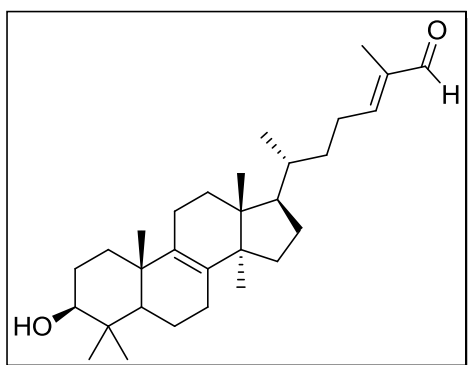
to completion, with the remainder accounting for residual starting material and an unidentified by product displaying a triplet at 3.60 ppm. Purification required repeated column chromatography using 70:30 hexane:ethyl acetate, and yielded the desired product 5 α -lanost-8-ene-7,11 dione 3-ol **19** as a yellow solidifying oil (0.219 g, 90%); ν_{max} (KBr)/cm⁻¹: 3247, 1670; δ_{H} (300 MHz, CDCl₃) 0.80 (3H, s, 18-CH₃), 0.86 – 0.89 (12H, m, containing 4 × CH₃, 19-CH₃, 21-CH₃, 26-CH₃, 27-CH₃), 1.02 (3H, s), 1.08-1.21 (6H, m, 29-CH₃, 28-CH₃), 1.30 (3H, s, 30-CH₃), 2.61 (1H, d, *J* 16.0, 12 β -CH), 2.75 (1H, d, *J* 16.1, 12 α -CH), 2.89 (1H, dt, *J* 13.7, 3.6, 1 β -CH), 3.28 (1H, dd, *J* 11.1, 5.1, 3-CH); δ_{C} (75 MHz, CDCl₃) 15.5 (29-CH₃), 16.8 (18-CH₃), 17.5 (30-CH₃), 18.6 (21-CH₃), 22.5 (26-CH₃), 22.8 (27-CH₃), 24.0 (23-CH₂), 25.9 (28-CH₃), 27.4 (16-CH₂), 27.6 (2-CH₂), 27.9 (19-CH₃), 28.0 (25-CH), 32.2 (15-CH₂), 34.1 (1-CH₂), 36.2 (20-CH), 36.2 (6-CH₂), 36.4 (22-CH₂), 38.9 (4-quaternary C), 39.4 (24-

CH₂), 39.8 (10-quaternary C), 47.4 (13-quaternary C), 49.0 (14-quaternary C), 49.1 (5-CH), 50.2 (17-CH), 51.7 (12-CH), 77.7 (3-CH), 150.7 (8-quaternary C), 151.8 (9-quaternary C), 202.3 (11-quaternary C, C=O), 202.6 (7-quaternary C, C=O); m/z 457 [M + H]⁺ (22%), 279 (100); exact mass calculated for C₃₀H₄₈O₃ [M + H]⁺ 457.3637, found 457.3690.

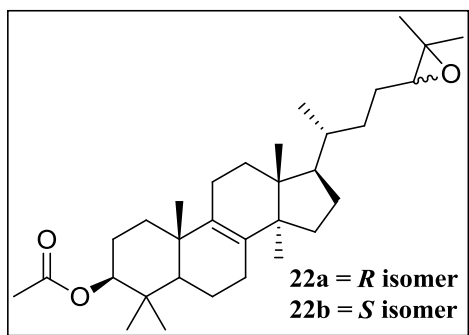
Method B⁶:

70% ^tButyl hydroperoxide (0.70 mL, 7.26 mM) was added dropwise to a stirring solution of an inseparable mixture of lanosterol **6** and dihydrolanosterol **7** (1:1) (0.100 g, 0.23 mM) and ruthenium trichloride (0.0007 g, 0.003 mM) in hexane (20 mL). The reaction solution was stirred at room temperature for 6 h. Saturated aqueous sodium bicarbonate (20 mL) was added to the reaction solution. The solution was then washed with sodium thiosulfate (2 × 20 mL) and brine (20 mL). The organic layer was dried over magnesium sulfate and concentrated under reduced pressure to yield the crude product (0.086 g). Analysis of the ¹H NMR spectrum of the crude material showed only starting materials present, with no evidence of formation of the desired product. Likewise, no product formation was observed upon analysis of the carbonyl region of the ¹³C NMR spectrum.

Attempted Allylic Oxidation using Selenium Dioxide Lanost-8-ene-27-al **20**



An inseparable mixture of lanosterol **6** and dihydrolanosterol **7** (0.200 g, 0.47 mM) (1:1) was dissolved in 95% ethanol (25 mL) and selenium dioxide (0.052 g, 0.47 mM) was added. The resulting solution was heated at reflux for 2 h.⁷ The reaction mixture was diluted with ethyl acetate (20 mL) and filtered through a bed of Celite[®]. The organic solution was washed with brine (2 × 20 mL), dried over magnesium sulfate and concentrated under reduced pressure to yield the crude product as a yellow oil. Analysis of the ¹H NMR spectrum of the crude material showed a majority of starting material, with evidence of the formation of ~6% of the desired product **20**, due to a broad singlet at 9.34 ppm.

3 β -Acetoxy-5 α -24-(*R,S*)-25-epoxy-lanost-8-ene^{1,2,8} **22a and **22b****

A 50:50 mixture of 3 β -acetoxy-5 α -lanosta-8,24-diene **10** and 3 β -acetoxy-5 α -lanost-8-ene **11** (4.160 g, 8.88 mM) was dissolved in dichloromethane (125 mL). *m*-CPBA (1.04 g, 6.04 mM), and sodium hydrogen carbonate (0.79 g, 9.41 mM) powders were mixed together. Half of this mixture was added at room temperature over 30 min, followed by the second half

at 0 °C over 30 min. The reaction mixture was stirred at 0 °C for 1 hour. The reaction mixture was filtered and washed with 10% sodium hydrogen carbonate (4 \times 50 mL) and brine (1 \times 50 mL). The organic layer was dried over magnesium sulfate and concentrated under reduced pressure to yield the crude product as a 1:1 mixture of 3 β -acetoxy-5 α -24-*R,S*-25-epoxy-lanost-8-ene **22a** and **22b** and 3 β -acetoxy-5 α -lanost-8-ene **11** (3.562 g). Purification by column chromatography using gradient elution with hexane:ethyl acetate led to the isolation of the desired product **22a** and **22b** as an off white solid as the second fraction from the column (1.600 g, 75%); mp 184 – 187 °C (literature,⁹ 171 – 172 °C); ν_{max} (KBr)/cm⁻¹ 2953, 1735, 1716; m/z (ESI⁺) 485 [M + H]⁺; exact mass calculated for C₃₂H₅₂O₃ [M + H]⁺ 485.3995, found 485.3988. δ_{H} (300 MHz, CDCl₃) 0.69 (3H, s, 18-CH₃), 0.88 (6H, s, 28-CH₃ and 29-CH₃), 0.91 – 0.95 (5H, m), 1.00 (3H, s, 19-CH₃), 1.11 – 1.15 (1H, m, 5-CH), 1.17 – 1.21 (2H, m), 1.27 (3H, s, 27-CH₃), 1.31 (3H, s, 26-CH₃), 1.48 – 2.10 (20H, m), 2.05 (3H, s, OAc, CH₃), 2.69 (1H, t, *J* 6.1, 24-CH for both α and β), 4.50 (1H, dd, *J* 11.2, 4.7, 3-CH).

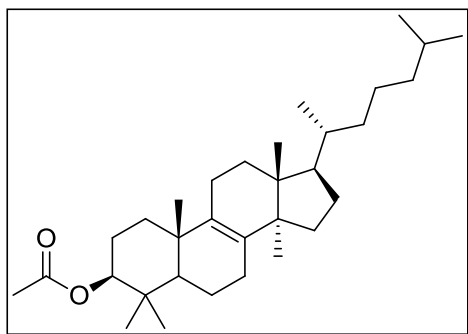
Peaks in the ¹H NMR spectrum are indistinguishable for the *R* and *S* isomers. However, clear differences can be deciphered in the ¹³C NMR spectrum. Key distinctions between the *R* and *S* isomers are seen at C-12, C-16, C-17, C-20, C-22, C-23, C-24, C-25, and C-27.

24-*R* isomer² **22a**

δ_{C} (75 MHz, CDCl₃) 15.7 (18-CH₃), 16.5 (CH₃), 18.1 (6-CH₂), 18.73 (27-CH₃), 19.2 (19-CH₃), 21.0 (11-CH₂), 21.3 (OAc, CH₃), 24.2 (30-CH₃), 24.2 (2-CH₂), 24.9 (26-CH₃), 25.61 (23-CH₂), 26.4 (7-CH₂), 27.9 (28-CH₃), 28.22 (16-CH₂), 30.8 (15-CH₂), 30.90 (12-CH₂), 32.60 (22-CH₂), 35.3 (1-CH₂), 36.21 (20-CH), 36.9 (10-quaternary C), 37.8 (4-quaternary C), 44.5 (13-quaternary C), 44.9 (14-quaternary C), 50.27 (17-CH), 50.5 (5-CH), 58.41 (25-quaternary C), 64.79 (24-CH), 80.9 (3-CH), 134.3 (9-quaternary C), 134.4 (8-quaternary C), 171.0 (OAc, quaternary C).

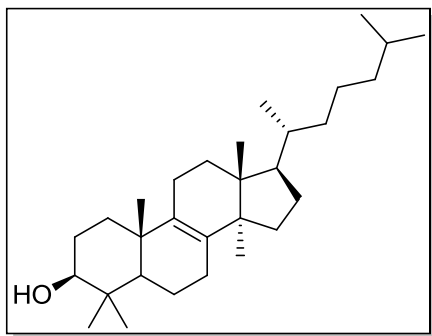
24-S isomer² 22b

δ_C (75 MHz, $CDCl_3$) 15.7 (18- CH_3), 16.5 (CH_3), 18.1 (6- CH_2), 18.66 (27- CH_3), 19.2 (19- CH_3), 21.0 (11- CH_2), 21.3 (OAc, CH_3), 24.2 (30- CH_3), 24.2 (2- CH_2), 24.9 (26- CH_3), 25.91 (23- CH_2), 26.4 (7- CH_2), 27.9 (28- CH_3), 28.17 (16- CH_2), 30.8 (15- CH_2), 30.95 (12- CH_2), 32.80 (22- CH_2), 35.3 (1- CH_2), 36.34 (20-CH), 36.9 (10-quaternary C), 37.8 (4-quaternary C), 44.5 (13-quaternary C), 44.9 (14-quaternary C), 50.38 (17-CH), 50.5 (5-CH), 58.14 (25-quaternary C), 64.94 (24-CH), 80.9 (3-CH), 134.3 (9-quaternary C), 134.4 (8-quaternary C), 171.0 (OAc, quaternary C).

3 β -Acetoxy-5 α -lanost-8-ene^{1,2} 11

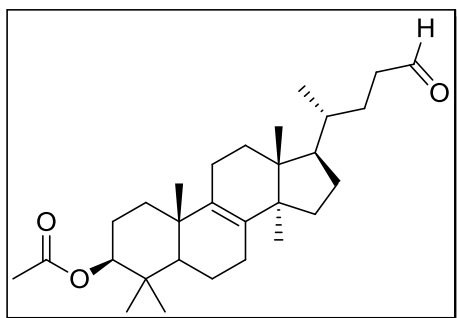
The first fraction from the column described above was 3 β -Acetoxy-5 α -lanost-8-ene **11**, isolated as a white solid with minor agnosterol impurity (<5%): mp 104 – 106 °C (literature,¹⁰ 119 – 121 °C); $\nu_{max}(KBr)/cm^{-1}$ 2927, 1737; δ_H (300 MHz, $CDCl_3$) 0.69 (3H, s, 18- CH_3), 0.85 – 0.90 (18H, m, containing 21- CH_3 , 26- CH_3 , 27- CH_3 , 28- CH_3 , 29- CH_3 , 30- CH_3),

1.01 (3H, s, 19- CH_3), 1.13 – 2.00 (25H, m, containing 5-CH, 17-CH, 20-CH, 25-CH), 2.05 (3H, s, OAc, CH_3), 4.50 (3H, dd, J 11.2, 4.6, 3-CH); δ_C (75 MHz, $CDCl_3$) 15.8 (18- CH_3), 16.5 (29- CH_3), 18.1 (6- CH_2), 18.7 (21- CH_3), 19.2 (19- CH_3), 21.0 (11- CH_2), 21.4 (OAc, CH_3), 22.6 (26- CH_3), 22.9 (27- CH_3), 24.12 (23- CH_2 or 2- CH_2), 24.18 (30- CH_3), 24.27 (23- CH_2 or 2- CH_2), 26.4 (7- CH_2), 27.9 (28- CH_3), 28.0 (25-CH), 28.2 (16- CH_2), 29.7 (CH_2), 30.8 (15- CH_2), 31.0 (12- CH_2), 35.3 (1- CH_2), 36.5 (22- CH_2), 36.9 (20-CH), 37.8 (4-quaternary C), 39.5 (24- CH_2), 44.5 (13-quaternary C), 49.8 (14-quaternary C), 50.5 (5-CH), 81.0 (3-CH), 134.2 (9-quaternary C), 134.5 (8-quaternary C), 171.1 (OAc, C=O).

3 β -Hydroxy-5 α -lanost-8-ene^{1,2} **7**

10% potassium hydroxide in ethanol (6 mL) was added to 3 β -acetoxy-5 α -lanost-8-ene **11** (0.200 g, 0.42 mM). The solution was stirred at reflux for 2 h and allowed to cool to room temperature. The resulting mixture was poured onto ice cold water (20 mL) and left to stand for 1 h. The solution was filtered through a Büchner funnel to yield the crude product as an off white solid (0.141 g).

Purification by column chromatography on silica gel (hexane:ethyl acetate 90:10) yielded the title compound **7** as a white solid (0.106 g, 59%): mp 125 – 128 °C (literature,¹¹ 125 – 125.5 °C); Found C, 83.53; H, 12.13. C₃₀H₅₂O requires C, 84.04; H, 12.23%; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3308, 2953; δ_{H} (300 MHz, CDCl₃) 0.69 (3H, s, 18-CH₃), 0.75 – 0.93 [15H, m, containing 21-CH₃, 26-CH₃, 27-CH₃, 30-CH₃, and 0.81 (3H, s, 29-CH₃)], 0.98 (3H, s, 19-CH₃), 1.00 (3H, s, 28-CH₃), 1.06 – 1.07 (1H, m, H-5), 1.10 – 2.06 (25H, m, containing 17-CH, 20-CH, 25-CH), 3.23 (1H, dd, *J* 11.3, 4.7, H-3); δ_{C} (75 MHz, CDCl₃) 15.4 (29-CH₃), 15.8 (18-CH₃), 18.3 (6-CH₂), 18.7 (21-CH₃), 19.2 (19-CH₃), 21.0 (11-CH₂), 22.6 (26-CH₃), 22.9 (27-CH₃), 24.1 (23-CH₂), 24.3 (30-CH₃), 26.5 (7-CH₂), 27.8 (2-CH₂), 27.97 (28-CH₃), 28.02 (25-CH), 28.2 (16-CH₂), 29.7 (CH₂), 30.9 (15-CH₂), 31.0 (12-CH₂), 35.6 (1-CH₂), 36.5 (20-CH₂ and 22-CH₂ possibly overlapping signals), 37.0 (10-quaternary C), 38.9 (4-quaternary C), 39.5 (24-CH₂), 44.5 (13-quaternary C), 49.8 (14-quaternary C), 50.4 (5-CH), 50.5 (17-CH), 79.0 (3-CH), 134.3 (9-quaternary C), 134.4 (8-quaternary C).

3 β -Acetoxy-5 α -4,4,14-trimethyl-chole-8-ene-24-al¹² **21**

A ~1:1 mixture of isomers 3 β -acetoxy-5 α -24*R,S*,25-epoxy-lanost-8-ene **22a** and **22b** (2.105 g, 4.3 mM) was added to a stirring solution of periodic acid (1.192 g, 6.2 mM) in diethyl ether (400 mL) at 25 °C. The solution was stirred under a blanket of nitrogen for 10 min. Water (200 mL) was added and the ether layer

was separated and washed with water (2 \times 100 mL). The organic layer was dried with magnesium sulfate and concentrated under reduced pressure to yield the crude product as an off white solid (1.73 g). Purification by column chromatography on silica gel (hexane:ethyl acetate 90:10) yielded the title compound **21** as a white solid (0.224 g, 12%. 88% of the material was of \geq 95% purity and synthetically useful). mp 153 – 156 °C (literature,⁹ 155 – 156 °C), ν_{max} (KBr)/cm⁻¹ 1717, 1735, 2952; δ_{H} (300 MHz, CDCl₃) 0.69 (3H, s, 18-CH₃), 0.80 – 0.95 (12H, m, containing 21-CH₃, 28-CH₃, 29-CH₃, 30-CH₃), 1.00 (3H, s, 19-CH₃), 1.15 – 2.00 (22H, m, containing 17-CH, 20-CH), 2.05 (3H, s, Ac-CH₃), 2.31-2.52 (2H, m, H-23), 4.50 (1H, dd, *J* 11.2, 4.7, 3-CH), 9.77 (1H, t, *J* 1.9, 24-CH); exact mass calculated for C₂₉H₄₆O₃ [M + H]⁺ 443.3525, found 443.3193.

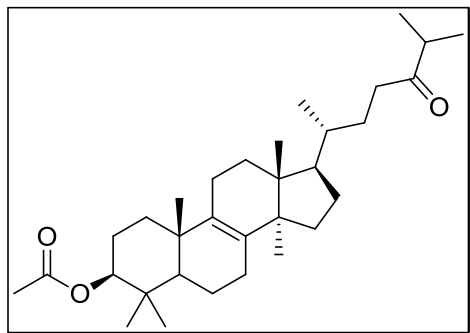
¹³C NMR data obtained 3 months after ¹H NMR data, in which time sample decomposition may have occurred.

δ_{C} (75 MHz, CDCl₃) 15.8 (18-CH₃), 16.5 (29-CH₃), 18.1 (6-CH₂), 18.4 (21-CH₃), 19.2 (19-CH₃), 21.0 (11-CH₂), 21.3 (OAc, CH₃), 24.2 (2-CH₂), 24.2 (30-CH₃), 26.4 (7-CH₂), 28.0 (28-CH₃), 28.1 (22-CH₂ or 23-CH₂), 28.2 (16-CH₂), 30.8 (15-CH₂), 31.0 (12-CH₂), 35.3 (1-CH₂), 36.0 (20-CH), 41.1 (23-CH₂ or 22-CH₂), 50.3 (CH), 50.5 (5-CH), 80.3 (3-CH), 134.3 (9-quaternary C), 134.4 (8-quaternary C), 171.1 (OAc, C=O), 178.9 (C=O), 203.3 (24-CH, C=O);

This reaction to synthesise the key aldehyde intermediate was carried out numerous times. In another attempt of this reaction, carried out on a separate occasion, a minor by product was isolated from the column. Spectral details and experimental quantities used are described below.

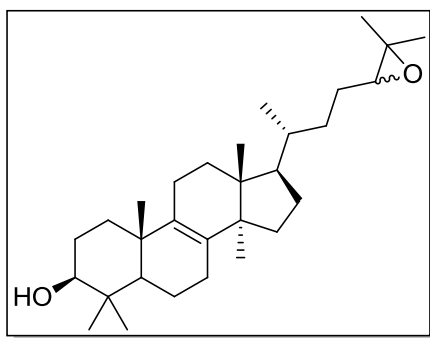
A by-product of the reaction to synthesise **21** was also isolated. Quantities used in the reaction on this occasion are as follows: 3 β -Acetoxy-24*R,S*,25-epoxy-5 α -lanost-8-ene **22a** and **22b** (1.63 g, 3.4 mM), periodic acid (1.1 g, 4.8 mM), diethyl ether (50 mL). Experimental procedure thereafter is as described above for the synthesis of **21**. By-product **30** was isolated as a yellow solid (0.016 g, 1%). Spectral details are described below.

3 β -Acetoxy-5 α -lanost-8-ene-24-one¹³ **30**



mp 88 – 90 °C (literature,¹³ 136 – 137 °C); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 2954, 1735, 1717; δ_{H} (300 MHz, CDCl_3) 0.68 (3H, s, 18- CH_3), 0.70 – 2.13 [45H, m, containing 21- CH_3 , 28- CH_3 , 29- CH_3 , 30- CH_3], 1.00 (3H, s, 19- CH_3), 1.09 (3H, d, J 6.9, 26- CH_3 or 27- CH_3), 2.05 (3H, s, OAc, CH_3), 2.26 – 2.69 (2H, m, 23- CH_2), 4.50 (1H, dd, J 4.7, 11.2, 3-CH); δ_{C} (75

MHz, CDCl_3) 15.8 (30- CH_3), 16.5 (18- CH_3), 18.1 (6- CH_2), 18.3, 18.4, and 18.5 (21- CH_3 , 26- CH_3 and 27- CH_3), 19.2 (19- CH_3), 21.0 (11- CH_2), 21.3 (OAc, CH_3), 24.16 (2- CH_2), 24.23 (28- CH_3), 26.4 (7- CH_2), 27.9 (29- CH_3), 28.1 (16- CH_2), 30.1 (23- CH_2), 30.8, 31.0 (12- CH_2 and 15- CH_2), 35.3 (1- CH_2), 36.1 (20-CH), 36.9 (10-quaternary C), 37.5 (22- CH_2), 37.8 (4-quaternary C), 40.8 (25-CH), 44.5 (13-quaternary C), 49.8 (14-quaternary C), 50.4 (5-CH or 17-CH), 50.5 (5-CH or 17-CH), 80.9 (3-CH), 134.3 (8-quaternary C or 9-quaternary C), 134.5 (8-quaternary C or 9-quaternary C), 171.0 (quaternary C, OAc, C=O), 215.4 (24-quaternary C, C=O).

3-Hydroxy-24(*R,S*)-25-epoxy-5 α -lanost-8-ene^{1,2,8} **23a and **23b****

3-Hydroxy-5 α -lanost-8-ene **7** and 3-hydroxy-5 α -lanost-8,24-diene **6** (50:50) (0.500 g, 1.2 mM) was dissolved in dichloromethane (20 mL) and stirred at room temperature. *m*-CPBA (0.137 g, 0.80 mM) and sodium bicarbonate (0.104 g, 1.24 mM) powders were mixed together. Half of this mixture was added to the stirring solution at room temperature and half was added at 0 °C, over 30 minute periods respectively. The reaction mixture was stirred at 0 °C for 1 h. The reaction mixture was filtered and washed with sodium bicarbonate (4 \times 25 mL) and brine (1 \times 25 mL). The organic layer was dried over magnesium sulfate and concentrated under reduced pressure to yield the crude product as an off white solid (0.354 g). ¹H NMR analysis showed that the majority of the crude material consisted of a 50:50 mixture of unreacted 3-hydroxy-5 α -lanost-8-ene **7** and 3-hydroxy-24,25-epoxy-5 α -lanost-8-ene **23a** and **23b** as well as trace steroid and aromatic impurities. Purification by column chromatography on silica gel (hexane:ethyl acetate 90:10) yielded the title compounds as a white solid in 16% yield: mp 125 – 126 °C (literature,¹⁴ 123 – 124 °C); ν_{max} (KBr)/cm⁻¹ 3482, 2956, 1030; *m/z* (ESI⁺) 443 [M + H]⁺ (17%), 267 (100); exact mass calculated for C₃₀H₅₁O₂ [M + H]⁺ 443.3889, found 443.3888.

δ_{H} (300 MHz, CDCl₃) 0.70 (3H, s, 18-CH₃), 0.81 (3H, s, 29-CH₃), 0.88 (3H, s, 30-CH₃), 0.92 (3H, d, *J* 6.0, 21-CH₃), 0.98 (3H, s, 19-CH₃), 1.00 (3H, s, 28-CH₃), 1.03 – 1.26 (4H, m), 1.27 (3H, s, 27-CH₃), 1.31 (3H, s, 26-CH₃), 1.39 – 2.06 (20H, m), 2.69 (1H, t, *J* 6.1, 24-CH), 3.23 (1H, dd, *J* 11.3, 4.6, 3-CH);

Peaks in the ¹H NMR spectrum are indistinguishable for the *R* and *S* isomers. However, clear differences can be deciphered in the ¹³C NMR spectrum. Key distinctions between the *R* and *S* isomers are seen at C-12, C-16, C-17, C-20, C-21, C-22, C-23, C-24, C-25, and C-27.

24-*R* isomer^{1,2} **23a**

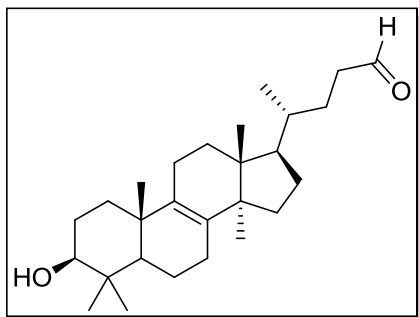
δ_{C} (75 MHz, CDCl₃) 15.4 (29-CH₃), 15.8 (18-CH₃), 18.3 (6-CH₂), 18.65 (21-CH₃), 18.75 (27-CH₃), 19.1 (19-CH₃), 21.0 (11-CH₂), 24.2 (30-CH₃), 24.9 (26-CH₃), 25.6 (23-CH₂), 26.5 (7-CH₂), 27.9 (2-CH₂), 28.0 (28-CH₃), 28.24 (16-CH₂), 30.8 (15-CH₂), 30.98 (12-CH₂), 32.62 (22-CH₂), 35.6 (1-CH₂), 36.22 (20-CH), 37.0 (10-quaternary C), 38.9 (4-quaternary C), 44.5 (13-quaternary C), 49.8 (14-quaternary C), 50.29, 50.38, 50.42 (CH due to C-5 or C-17 for *R*

and *S* isomers), 58.40 (25-quaternary C), 64.79 (24-CH), 79.0 (3-CH), 134.4 (8-quaternary C), 134.5 (9-quaternary C).

24-*S* isomer^{1,2} **23b**

δ_{C} (75 MHz, CDCl_3) 15.4 (29- CH_3), 15.8 (18- CH_3), 18.3 (6- CH_2), 18.58 (21- CH_3), 18.67 (27- CH_3), 19.1 (19- CH_3), 21.0 (11- CH_2), 24.2 (30- CH_3), 24.9 (26- CH_3), 25.9 (23- CH_2), 26.5 (7- CH_2), 27.9 (2- CH_2), 28.0 (28- CH_3), 28.19 (16- CH_2), 30.8 (15- CH_2), 31.00 (12- CH_2), 32.81 (22- CH_2), 35.6 (1- CH_2), 36.33 (20-CH), 37.0 (10-quaternary C), 38.9 (4-quaternary C), 44.5 (13-quaternary C), 49.8 (14-quaternary C), 50.29, 50.38, 50.42 (CH due to C-5 or C-17 for *R* and *S* isomers), 58.11 (25-quaternary C), 64.93 (24-CH), 79.0 (3-CH), 134.4 (8-quaternary C), 134.5 (or 9-quaternary C).

3 β -Hydroxy-5 α -4,4,14-trimethyl-chole-8-ene-24-al¹² **25**



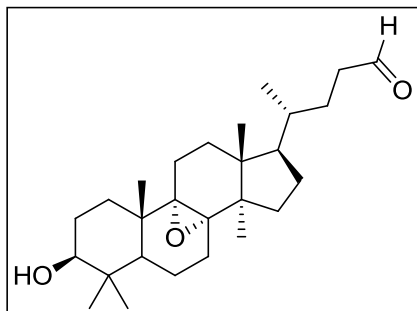
Epoxide 3 β -hydroxy-5 α -24*R,S*,25-epoxy-lanost-8-ene **23a** and **23b** (1.835 g, 4.1 mM) was added to a stirring solution of periodic acid (1.351 g, 5.9 mM) in diethyl ether (350 mL) at 25 °C. The solution was stirred under a blanket of nitrogen for 10 min. Water (200 mL) was added and the ether layer was separated and washed with

water (2 \times 100 mL). The organic layer was dried with magnesium sulfate and concentrated under reduced pressure to yield the desired product **25** as an off white solid (1.21 g, 73%).

$\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3520, 2948, 1715; δ_{H} (300 MHz, CDCl_3) 0.69 (3H, s, 18- CH_3), 0.81 (3H, s, 29- CH_3), 0.88 (3H, s, 30- CH_3), 0.91 (3H, d, *J* 5.9, 21- CH_3), 0.98 (3H, s, 19- CH_3), 1.00 (3H, s, 28- CH_3), 1.02 – 2.24 (30H, m), 2.25 – 2.55 (2H, m, 23- CH_2), 3.25 (1H, dd, *J* 4.7, 11.2, 3-CH), 9.77 (1H, t, *J* 1.9, 24-CH); δ_{C} (75 MHz, CDCl_3) 15.4 (29- CH_3), 15.8 (18- CH_3), 18.3 (6- CH_2), 18.4 (21- CH_3), 19.2 (19- CH_3), 21.0 (11- CH_2), 24.2 (30- CH_3), 26.5 (7- CH_2), 27.9 (2- CH_2), 28.0 (28- CH_3), 28.2 (16- CH_2), 28.3 (22- CH_2), 30.8 (15- CH_2), 31.0 (12- CH_2), 35.6 (1- CH_2), 36.1 (20-CH), 37.1 (10-quaternary C), 38.9 (4-quaternary C), 41.2 (23- CH_2), 44.6 (13-

quaternary C), 49.8 (14-quaternary C), 50.3 (5-CH), 50.4 (17-CH), 79.0 (3-CH), 134.3 (9-quaternary C), 134.5 (8-quaternary C), 203.2 (24-CH, C=O);

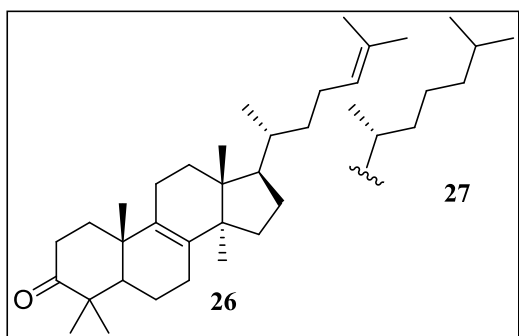
3 β -Hydroxy-5 α -4,4,14-trimethyl-chol-8,9-epoxy-24-al **72**



m-CPBA (0.362 g, 2.09 mM) was added to a stirring solution of aldehyde **25** (0.600 g, 1.49 mM) in dichloromethane (50 mL). The solution was stirred at room temperature over 48 h. The solution was then filtered and washed with NaHCO₃ (2 × 50 mL) which led to the formation of emulsions. Extensive washing of the

emulsion with brine and drying of the organic layer with magnesium sulfate led to the formation of the desired product **72** (0.947 g) which contains residual *m*-CPBA. The product could not be purified after several recrystallisation attempts, and is ~ 30% pure. δ_{H} (300 MHz, CDCl₃) 0.60 – 2.16 [48H, m, containing 0.77 (3H, s, 18-CH₃), 0.88 (3H, s, 30-CH₃), 1.12 (3H, s, 19-CH₃) 21-CH₃, 28-CH₃, 29-CH₃], 2.35 – 2.55 (2H, m, 23-CH₂), 3.21 (1H, dd, *J* 11.2, 4.0, 3-CH), 9.77 (1H, br s, 24-CH); δ_{C} (300 MHz, CDCl₃) characteristic epoxide peaks 68.1 (8-quaternary C) and 70.7 (9-quaternary C).

3-Keto-24,25-dihydro-lanost-8-ene **27** and 3-keto-lanost-8-ene¹⁵ **26**



An inseparable mixture of lanosterol **6** and dihydrolanosterol **7** in a 50:50 ratio (0.300 g, 0.70 mM) was stirred in *t*-butanol (60 mL). Iodine (0.714 g, 2.81 mM, 4 eq.) and potassium carbonate (0.388 g, 2.81 mM, 4 eq.) was added and the solution was stirred at reflux for 36 h.

After this time, the solution was removed from the oil bath, allowed to cool to room temperature, then further cooled to 0 °C in an ice bath. Saturated sodium thiosulfate (50 mL) was added at 0 °C and the solution was extracted with ethyl acetate (3 × 30 mL). The organic layer was washed with brine (50 mL) and dried over MgSO₄ and concentrated under reduced pressure to yield the crude product mixture of **27** and **26** as a brown solid (0.305 g). Purification by column chromatography (gradient 95:5 to 90:10

hexane:ethyl acetate) yielded the desired mixture of 3-keto-24,25-dihydro-lanost-8-ene **27** and 3-keto-lanost-8-ene **26** in a 40:60 ratio (0.106 g).

Note: Mass yields ranged from 35% to 57% upon increase of the reaction time to 48 h, to 50% upon increase of the I_2 and K_2CO_3 to 4 equivalents and shortening of reaction time to 36 h, as per the reaction described above,. Spectra below described as a mixture of compounds.

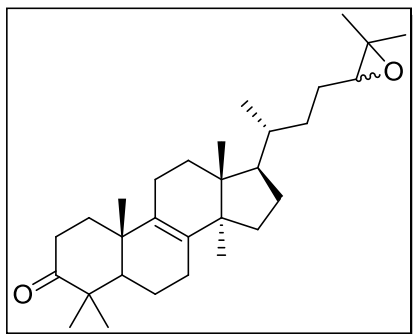
3-Keto-24,25-dihydro-lanost-8-ene **27**

δ_H (300 MHz, $CDCl_3$) 0.72 (3H, s, 18- CH_3), 0.78 – 2.34 [45H, m containing 0.80 – 0.94 (9H, m, containing 21- CH_3 , 30- CH_3), 1.07 (3H, s, 29- CH_3), 1.09 (3H, s, 28- CH_3), 1.12 (3H, s 19- CH_3), 1.15 – 2.32 (27H, m)] 2.35 – 2.50 (1H, m, one of 2- CH_2), 2.50 – 2.65 (1H, m, one of 2- CH_2).

3-keto-lanost-8-ene **26**

δ_H (300 MHz, $CDCl_3$) 0.72 (3H, s, 18- CH_3), 0.78 – 2.34 [42H, m containing 0.80 – 0.94 (9H, m, containing 21- CH_3 , 30- CH_3), 1.07 (3H, s, 29- CH_3), 1.09 (3H, s, 28- CH_3), 1.12 (3H, s 19- CH_3), 1.15 – 2.32 (27H, m)] 2.35 – 2.50 (1H, m, one of 2- CH_2), 2.50 – 2.65 (1H, m, one of 2- CH_2) 5.10 (1H, t, J 7.1, 24-CH).

3-Keto-24(*R,S*)-25-epoxy-5 α -lanost-8-ene **24**



3-Keto-5 α -lanost-8-ene **26** and 3-keto-24,25-dihydro-lanost-8-ene **27** (60:40) (0.106 g, 0.23 mM) was dissolved in dichloromethane (15 mL). *m*-CPBA (0.028 g, 0.16 mM) and sodium bicarbonate (0.021 g, 0.25 mM) powders were mixed together. Half of this mixture was added to the stirring solution at room temperature and half was added at 0 °C, over 30 minute periods respectively.^{1,8} The

reaction mixture was stirred at 0 °C for 1 h. The reaction mixture was filtered and washed with 10% sodium bicarbonate (4 × 25 mL) and brine (1 × 25 mL). The organic layer was dried over magnesium sulfate and concentrated under reduced pressure to yield the crude product as a mixture of unreacted **27** (spectral details described separately below) and stereoisomers **24a** and **24b** as an off white solid mixture (0.102 g). Purification by chromatography on silica gel hexane:ethyl acetate (95:5) yielded the desired products **24a** and **24b** as a yellow solid mixture (0.024 g, 44%): mp 104 – 106 °C; $\nu_{max}(KBr)/cm^{-1}$ 2951, 1708;

δ_{H} (300 MHz, CDCl_3) 0.73 (3H, s, 18- CH_3), 0.89 (3H, s, 30- CH_3), 0.92 (3H, d, J 6.1, 21- CH_3), 1.06 (3H, s, 29- CH_3), 1.10 (3H, s, 28- CH_3), 1.12 (3H, s, 19- CH_3), 1.20 – 2.30 [25H, m, containing 1.27 (3H, s, 27- CH_3), 1.31 (3H, s, 26- CH_3)], 2.32 – 2.44 (1H, m, one of 2- CH_2), 2.46 – 2.63 (1H, m, one of 2- CH_2), 2.69 (1H, t, J 6.2, 24-CH); m/z (ESI^+) 441 [$\text{M} + \text{H}$] $^+$ (48%), 267 (100); exact mass calc for $\text{C}_{30}\text{H}_{48}\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 441.3688, found 441.3750.

Peaks in the ^1H NMR spectrum are indistinguishable for the *R* and *S* isomers. However, clear differences can be deciphered in the ^{13}C NMR spectrum. Key distinctions between the *R* and *S* isomers are seen at C-16, C-17, C-20, C-22, C-23, C-24 and C-25

***R* isomer 24a**

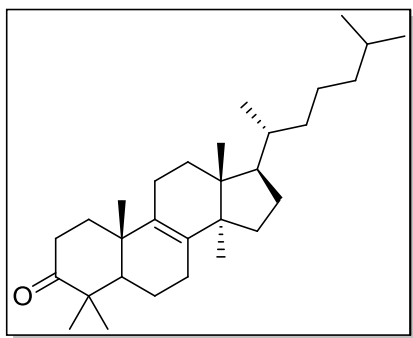
δ_{C} (75 MHz, CDCl_3) 15.9 (18- CH_3), 18.5 (21- CH_3), 18.7 (27- CH_3), 18.8 (19- CH_3), 19.4 (6- CH_2), 21.1 (11- CH_2), 21.3 (29- CH_3), 24.3 (30- CH_3), 24.9 (26- CH_3), 25.6 (15- CH_2), 25.61 (23- CH_2), 26.2 (28- CH_3), 26.3 (7- CH_2), 28.20 (16- CH_2), 30.9 (12- CH_2), 32.59 (22- CH_2), 34.6 (2- CH_2), 36.1 (1- CH_2), 36.21 (20-CH), 36.9 (10-quaternary C), 44.5 (13-quaternary C), 47.4 (4-quaternary C), 49.9 (14-quaternary C), 50.27 (17-CH), 51.2 (5-CH), 58.44 (25-quaternary C), 64.78 (24-CH), 133.1 (8-quaternary C), 135.3 (9-quaternary C), 217.9 (3-quaternary C, C=O).

***S* isomer 24b**

δ_{C} (75 MHz, CDCl_3) 15.9 (18- CH_3), 18.5 (21- CH_3), 18.7 (27- CH_3), 18.8 (19- CH_3), 19.4 (6- CH_2), 21.1 (11- CH_2), 21.3 (29- CH_3), 24.3 (30- CH_3), 24.9 (26- CH_3), 25.6 (15- CH_2), 25.92 (23- CH_2), 26.2 (28- CH_3), 26.3 (7- CH_2), 28.16 (16- CH_2), 30.9 (12- CH_2), 32.76 (22- CH_2), 34.6 (2- CH_2), 36.1 (1- CH_2), 36.34 (20-CH), 36.9 (10-quaternary C), 44.5 (13-quaternary C), 47.4 (4-quaternary C), 49.9 (14-quaternary C), 50.38 (17-CH), 51.2 (5-CH), 58.14 (25-quaternary C), 64.94 (24-CH), 133.1 (8-quaternary C), 135.3 (9-quaternary C), 217.9 (3-quaternary C, C=O).

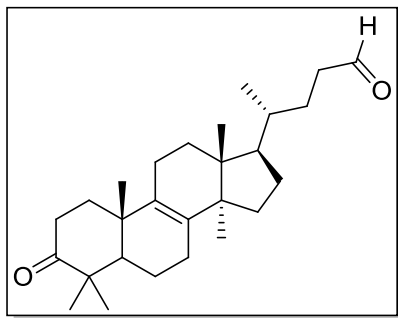
Method B:

3-Hydroxy-24(*R,S*)-25-epoxy-5 α -lanost-8-ene **23a** and **23b** (1:1) (0.100 g, 0.26 mM) was dissolved in t-butanol (50 mL). Iodine (0.115 g, 0.45 mM, 2 eq.) and potassium carbonate (0.062 g, 0.45 mM, 2 eq.) was added and the solution was stirred at reflux for 36 h. Experimental procedure thereafter as per described for the synthesis of **26** and **27**. Product was retrieved as a yellow oily solid (0.78 g) with ^1H NMR analysis showing ~10% product formation.

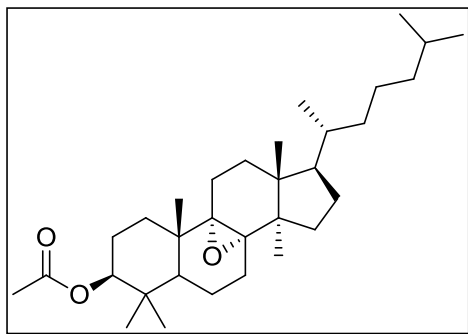
3-Keto-24,25-dihydro-lanost-8-ene¹⁵ 27

An inseparable 50:50 mixture of 3-keto-lanost-8-ene **26** and 3-keto-24,25-dihydro-lanost-8-ene **27** (0.97 g, 2.28 mM) was dissolved in 100 mL DCM. *m*CPBA (0.266 g, 1.55 mM) and sodium bicarbonate (0.203 g, 2.42 mM) powders were mixed together. Half of this mixture was added at room temperature over a 30 minute period. The second half was added at 0 °C over a 30 minute period.

The reaction solution was stirred at 0 °C for 1 h. The reaction mixture was filtered, washed with saturated sodium bicarbonate solution (2 × 50 mL) and brine (1 × 50 mL), dried over magnesium sulfate and concentrated under reduced pressure to yield the crude product as a mixture of 3-keto-24,25-dihydro-lanost-8-ene **27** and 3-keto-24(*R,S*)-25-epoxy-lanost-8-ene **24a** and **24b** (0.827 g). Purification required repeated column chromatography using 90:10 hexane: ethyl acetate, and yielded the desired product **27** as an off white solid (0.043 g, 9%) from the second column. 3-Keto-24(*R,S*)-25-epoxy-lanost-8-ene **24a** and **24b** (0.158 g) was isolated from the first column and contained impurities including aromatic contaminants. This material was not further purified. Spectral analysis of a pure sample of the mixture of isomers **24a** and **24b** is described above. The following analysis pertains to compound **27**: mp 117 – 120 °C (literature,¹⁶ 119 – 120 °C); ν_{max} (KBr)/cm⁻¹ 2956, 1708; δ_{H} (300 MHz, CDCl₃) 0.72 (3H, s, 18-CH₃), 0.82 – 0.92 (12H, m, containing 21-CH₃, 26-CH₃, 27-CH₃, 30-CH₃), 1.07 (3H, s, 29-CH₃), 1.09 (3H, s, 28-CH₃), 1.12 (3H, s 19-CH₃), 1.23 – 2.35 (24H, m), 2.37 – 2.49 (1H, m, one of 2-CH₂), 2.51 – 2.63 (1H, m, one of 2-CH₂); δ_{C} (75 MHz, CDCl₃) 15.8 (18-CH₃), 18.7 (19-CH₃), 18.7 (21-CH₃), 19.4 (6-CH₂), 21.1 (7-CH₂), 21.3 (29-CH₃), 22.6 (26-CH₃), 22.8 (27-CH₃), 24.1 (23-CH₂), 24.3 (30-CH₃), 26.2 (28-CH₃), 26.3 (11-CH₂), 28.0 (25-CH), 28.2 (16-CH₂), 30.91 (15-CH₂), 30.93 (12-CH₂), 34.6 (2-CH₂), 36.1 (1-CH₂), 36.4 (22-CH₂), 36.5 (20-CH), 36.9 (4-quaternary C), 39.5 (24-CH₂), 44.4 (13-quaternary C), 47.5 (10-quaternary C), 49.9 (14-quaternary C), 50.5 (17-CH), 51.2 (5-CH), 133.1 (8-quaternary C), 135.4 (9-quaternary C), 218.0 (3-quaternary C, C=O); exact mass calc for C₃₀H₅₁O [M + H]⁺ 427.3940, found 427.3931.

3-Keto-4,4,14-trimethyl-chol-8-ene-24-al¹⁵ 29

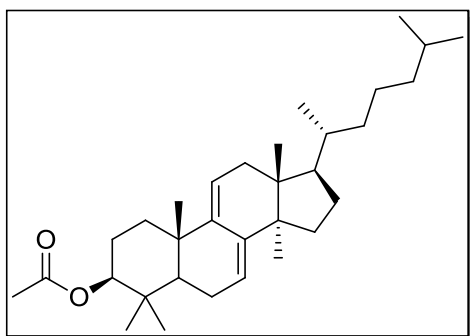
Periodic acid (0.078 g, 0.34 mM) was ground using a pestle and mortar and dissolved in diethyl ether (50 mL). 3-Keto-24(*R,S*)-25-epoxy-5 α -lanost-8-ene **24a** and **24b** (0.106 g, 0.24 mM) was added and the solution was stirred at 25 °C for 15 min. Water (20 mL) was added and the ether layer was separated. The ether layer was washed with water (2 \times 20 mL) and brine (1 \times 20 mL), dried over MgSO₄ and concentrated under reduced pressure to yield the crude product as an off white solid (0.076 g). Purification by column chromatography using 90:10 hexane: ethyl acetate yielded the desired product **29** as a white solid (0.015 g, 16% analytically pure sample, 84% of the material was of \geq 95% purity and synthetically useful): mp 111 – 114 °C (literature,¹⁷ 110 – 115 °C); ν_{max} (KBr)/cm⁻¹ 2948, 1719, 1707; δ_{H} (300 MHz, CDCl₃) 0.72 (3H, s, 18-CH₃), 0.87 – 0.94 (6H, m, containing 21-CH₃ and 30-CH₃), 1.07 (3H, s, 29-CH₃), 1.10 (3H, s, 28-CH₃), 1.17 (3H, s, 19-CH₃), 1.20 – 2.30 (20H, m), 2.36 – 2.63 (4H, m, containing 2-CH₂ and 23-CH₂), 9.78 (1H, t, *J* 1.8, 24-CH); δ_{C} (75 MHz, CDCl₃) 15.9 (29-CH₃), 18.4 (18-CH₃), 18.7 (21-CH₃), 19.4 (6-CH₂), 21.1 (11-CH₂), 21.3 (19-CH₃), 24.3 (30-CH₃), 26.2 (28-CH₃), 26.3 (7-CH₂), 28.1 (16-CH₂), 28.2 (22-CH₂), 30.86 (15-CH₂), 30.90 (12-CH₂), 34.6 (1-CH₂), 36.0 (20-CH), 36.1 (2-CH₂), 36.9 (4-quaternary C), 41.2 (23-CH₂), 44.5 (13-quaternary C), 47.4 (10-quaternary C), 49.9 (14-quaternary C), 50.3 (5-CH), 51.2 (17-CH), 133.2 (8-quaternary C), 135.2 (9-quaternary C), 203.2 (24-quaternary C, C=O), 217.9 (3-quaternary C, C=O); exact mass calc for C₂₇H₄₃O₂ [M + H]⁺ 399.3263, found 399.3257.

3 β -Acetoxy-lanost-8,9-epoxide^{1,2} 1

Dihydrolanosterol acetate **11** (0.400 g, 0.85 mM) was dissolved in dichloromethane (100 mL) and *m*-CPBA (0.205 g, 1.19 mM) was added. The reaction mixture was stirred at room temperature while the reaction progress was monitored by TLC analysis. After 4 h the reaction had gone ~ 50% to completion. A further 2.8 equivalents of *m*-CPBA was added, and the

solution was allowed to stir at room temperature for 72 h. After this time TLC analysis showed the main component of the reaction mixture to be the desired product **1**. The reaction mixture was then filtered, washed with 10% sodium bicarbonate (2×50 mL) and brine (1×50 mL), dried over MgSO_4 and concentrated under reduced pressure to yield the crude product as an off white solid (0.342 g). Purification by column chromatography using 90:10 hexane: ethyl acetate yielded the desired product **1** as a white solid (0.158 mg, 38%); mp $173 - 175$ °C (literature,¹⁸ $175 - 177$ °C); $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 2957, 1724; δ_{H} (300 MHz, CDCl_3) 0.77 (3H, s, 18- CH_3), 0.80 – 0.90 [18H, m, containing 0.82 (3H, s, 28- CH_3), 0.85 – 0.86 (6H, m, 26- CH_3 and 29- CH_3), 0.87 – 0.89 (9H, m, containing 21- CH_3 , 27- CH_3 and 30- CH_3)], 1.05 – 1.22 [6H, m, containing 1.14 (3H, s, 19- CH_3)], 1.23 – 1.44 (12H, m), 1.45 – 1.66 (6H, m), 1.67 – 1.79 (3H, m), 1.80 – 2.00 (4H, m), 2.03 (3H, s, OAc, CH_3), 4.46 (1H, dd, J 11.0, 4.2, 3-CH); δ_{C} (75 MHz, CDCl_3) 16.2 (29- CH_3), 16.3 (18- CH_3), 16.4 (6- CH_2), 17.1 (19- CH_3), 19.0 (21- CH_3), 19.9 (30- CH_3), 21.3 (OAc, CH_3), 21.5 (11- CH_2), 22.6 (26- CH_3), 22.8 (27- CH_3), 23.3 (7- CH_2), 23.5 (2- CH_2), 24.1 (23- CH_2), 26.8 (12- CH_2), 28.0 (25-CH), 28.3 (28- CH_3), 28.6 (16- CH_2), 29.7 (CH_2), 31.9 (15- CH_2), 32.5 (1- CH_2), 36.3 (20-CH), 36.4 (22- CH_2), 37.4 (4-quaternary C), 37.8 (10-quaternary C), 39.5 (24- CH_2), 41.9 (5-CH), 43.6 (13-quaternary C), 48.3 (17-CH), 48.8 (14-quaternary-C), 68.0 (8-quaternary C), 70.5 (9-quaternary C), 80.5 (3-CH), 170.8 (quaternary C, OAc, $\text{C}=\text{O}$); m/z (ESI^+) 487 [$\text{M} + \text{H}$]⁺ (30%), 469 (100); exact mass calculated for $\text{C}_{32}\text{H}_{55}\text{O}_3$ [$\text{M} + \text{H}$]⁺ 487.4151, found 487.4132.

3 β -Acetoxy-5 α -lanost-7,9(11)-diene **2**¹⁷

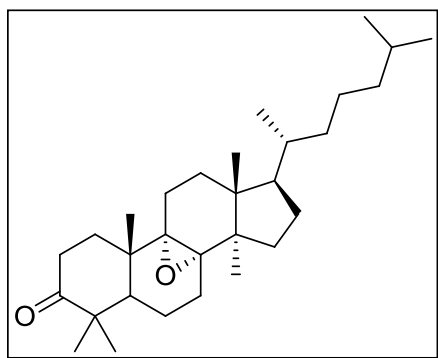


A mixture of epoxide 3 β -acetoxy-lanost-8,9-epoxide **1** (0.100 g, 0.21 mM) and *p*-toluenesulfonic acid (0.004 g, 0.02 mM) in toluene was stirred for 2.5 h at 60 °C. The reaction was quenched with triethylamine (3 μL , 0.02 mM) and filtered through celite. The celite was washed with toluene (15 mL) and the solvent was removed under reduced pressure to yield the crude

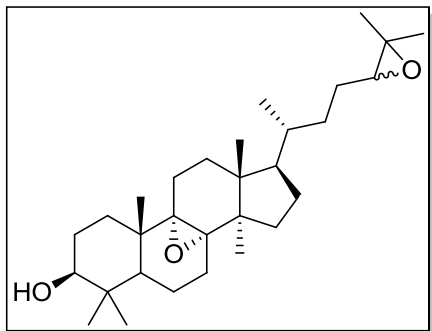
product as a white solid (0.087 g). Inspection of the ^1H NMR spectrum of the crude material showed an impurity with a characteristic splitting pattern, 4.46 ppm (0.5H, dd, J 11.0, 4.2),

which is characteristic of the 3-CH of the starting epoxide **1**. Purification required repeated column chromatography using 90: 10 hexane: ethylacetate. The desired compound **2** was obtained as a white solid (0.021 g, 22%); mp 168 – 171 °C (literature,¹⁹ 169 – 170°C); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 2945, 1735; δ_{H} (300 MHz, CDCl_3) 0.56 (3H, s, 18- CH_3), 0.80 – 0.91 [15H, m, containing 0.86 (3H, s, 26- CH_3), 0.87 (6H, s, 27- CH_3 and 30- CH_3), 0.88 (3H, s, 21- CH_3), 0.89 (3H, s, 28- CH_3)], 0.95 (3H, s, 29- CH_3), 1.01 (3H, s, 19- CH_3), 1.06 – 1.21 (4H, m containing 5-CH), 1.22 – 1.44 (7H, m, containing 20-CH), 1.46 – 1.82 (8H, m, containing 17-CH and 25-CH), 1.85 – 2.30 [5H, m, containing 2.06 (3H, s, OAc, CH_3)], 4.52 (1H, dd, J 11.1, 4.8, 3-CH), 5.34 (1H, br d, J 6.2, 11-CH), 5.46 (1H, br s, 7-CH); δ_{C} (75 MHz, CDCl_3) 15.7 (18- CH_3), 16.9 (29- CH_3), 18.5 (21- CH_3), 21.3 (OAc, CH_3), 22.6 (26- CH_3), 22.79 (19- CH_3), 22.83 (6- CH_2), 24.1 (23- CH_2), 24.3 (2- CH_2), 25.6 (30- CH_3), 27.9 (16- CH_2), 28.0 (25-CH), 28.1 (28- CH_3), 31.5 (15- CH_2), 35.4 (1- CH_2), 36.3 (20-CH), 36.4 (22- CH_2), 37.2 (10-quaternary C), 37.6 (4-quaternary C), 37.9 (12- CH_2), 39.5 (24- CH_2), 43.7 (13-quaternary C), 49.3 (5-CH), 50.3 (14-quaternary C), 51.1 (17-CH), 80.9 (3-CH), 116.6 (11-CH), 119.8 (7-CH), 142.9 (8-quaternary C), 145.6 (9-quaternary C), 170.9 (quaternary C, OAc, $\text{C}=\text{O}$); exact mass calculated for $\text{C}_{32}\text{H}_{52}\text{O}_2$ $[\text{M} + \text{H}]^+$ 469.4046, found 469.4030.

3-Keto-lanost-8,9-epoxide **28**



3-Keto-24,25-dihydro-lanost-8-ene **27** (0.250 g, 0.59 mM) was dissolved in dichloromethane (100 mL) and the reaction mixture was stirred at room temperature for 24 h. After this time the reaction mixture was filtered and washed with 10% sodium bicarbonate (2×50 mL) followed by brine (50 mL). The solvent was removed under reduced pressure to yield the crude product as an off white solid (0.209 g). The crude product was purified by repeated column chromatography using 90:10 hexane : ethylacetate as the eluant. Due to insufficient sample, further purification was not possible. The sample described contains minor amounts of residual *m*-CPBA. δ_{H} (300 MHz, CDCl_3) 0.78 (3H, s, 18- CH_3), 0.85 – 0.90 (17H, m, containing 21- CH_3 , 30- CH_3), 1.02 (3H, s, 29- CH_3), 1.04 (3H, s, 28- CH_3), 1.25 (3H, s 19- CH_3), 2.39 – 2.61 (2H, m, 2- CH_2).

3 β -Hydroxy-24(*R,S*)-25-epoxy-5 α -lanost-8,9-epoxide **38a and **38b****

A 1:1 mixture of lanosterol **6** and dihydrolanosterol **7** (1 g, 2.33 mM), were dissolved in dichloromethane (50 mL). *m*-CPBA (1.200 g, 7 mM) was added and the mixture was stirred at room temperature for 72 h. The resulting solution was filtered and washed with 10% aqueous sodium bicarbonate (2 \times 50 mL) and brine (1 \times 50 mL). The organic layer was dried over magnesium

sulfate and concentrated under reduced pressure to yield the crude product as a yellow solid (1.245 g) which contained trace amounts of unreacted alkenes as indicated by analysis of the ^{13}C NMR spectrum. Purification required repeated column chromatography using 90: 10 hexane: ethylacetate. The desired mixture of compounds **38a** and **38b** was obtained as a white solid (0.234 g, 60%); mp 156 – 158 $^{\circ}\text{C}$; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3271, 2961; δ_{H} (300 MHz, CDCl_3) 0.79 (6H, s, 18- CH_3 and 29- CH_3), 0.83 – 1.04 [10H, m, containing (3H, s, 30- CH_3), (3H, s, 21- CH_3) and (3H, s, 28- CH_3)], 1.12 (3H, s, 19- CH_3), 1.21 – 2.20 [28H, m, containing 1.26 (3H, s, 27- CH_3), 1.31 (3H, s, 26- CH_3)], 2.68 (1H, t, J 5.9, 24-CH), 3.21 (1H, br d, J 10.7, 3-CH); exact mass calc for $\text{C}_{30}\text{H}_{51}\text{O}_3$ [$\text{M} + \text{H}$] $^{+}$ 459.3838, found 459.3828.

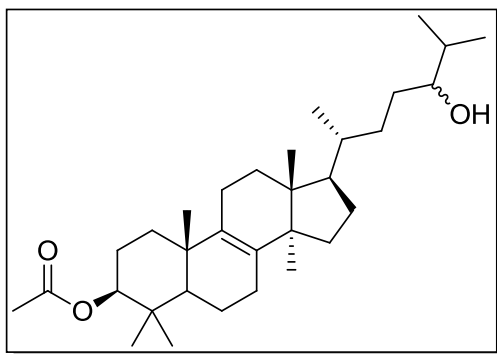
Peaks in the ^1H NMR spectrum are indistinguishable for the *R* and *S* isomers. However, clear differences can be deciphered in the ^{13}C NMR spectrum. Key distinctions between the 24-*R* and 24-*S* isomers are seen at C-16, C-20, C-21, C-22, C-23, C-24, C-25 and C-27. Signals pertaining to C-21 and C-27 of both *R* and *S* isomers could not be unambiguously assigned as these 4 peaks occur within 0.27 ppm of each other. All literature precedent indicates internal C-8,9 epoxide formation exclusively on the α face.^{2,8}

24-*R* isomer **38a**

δ_{C} (75 MHz, CDCl_3) 15.1 (29- CH_3), 16.2 (18- CH_3), 16.5 (6- CH_2), 17.0 (19- CH_3), 18.65, 18.76, 18.81, 18.92 (4 \times CH_3 , 21- CH_3 and 27- CH_3 for both *R* and *S* isomers), 19.9 (30- CH_3), 21.4 (11- CH_2), 23.4 (7- CH_2), 24.9 (26- CH_3), 25.5 (23- CH_2), 26.8 (12- CH_2), 27.1 (2- CH_2), 28.3 (28- CH_3), 28.46 (16- CH_2), 31.8 (15- CH_2), 32.5 (22- CH_2), 32.8 (1- CH_2), 36.0 (20-CH), 37.9 (10-quaternary C), 38.5 (4-quaternary C), 41.7 (5-CH), 43.6 (13-quaternary C), 48.2 (17-CH), 48.8 (14-quaternary C), 58.5 (25-quaternary C), 64.7 (24-CH), 68.1 (8-quaternary C), 70.7 (9-quaternary C), 78.4 (3-CH).

24-*S* isomer 38b

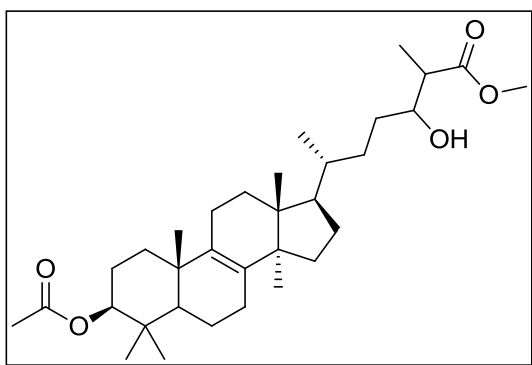
δ_C (75 MHz, $CDCl_3$) 15.1 (29- CH_3), 16.2 (18- CH_3), 16.5 (6- CH_2), 17.0 (19- CH_3), 18.65, 18.76, 18.81, 18.92 ($4 \times CH_3$, 21- CH_3 and 27- CH_3 for both *R* and *S* isomers), 19.9 (30- CH_3), 21.4 (11- CH_2), 23.4 (7- CH_2), 24.9 (26- CH_3), 25.9 (23- CH_2), 26.8 (12- CH_2), 27.1 (2- CH_2), 28.3 (28- CH_3), 28.50 (16- CH_2), 31.8 (15- CH_2), 32.7 (22- CH_2), 32.8 (1- CH_2), 36.2 (20-CH), 37.9 (10-quaternary C), 38.5 (4-quaternary C), 41.7 (5-CH), 43.6 (13-quaternary C), 48.1 (17-CH), 48.8 (14-quaternary C), 58.2 (25-quaternary C), 64.9 (24-CH), 68.1 (8-quaternary C), 70.7 (9-quaternary C), 78.4 (3-CH).

3 β -Acetoxy-5 α -lanost-8-ene-24(*R,S*)-ol²⁰ 31a and 31b

All glassware to be used for the Grignard reaction was dried overnight at 150 °C prior to use. The reaction was carried out in an anhydrous atmosphere, using a blanket of nitrogen at all stages of reagent addition. Glassware joints were greased to avoid air entering the reaction system. 1-Bromo-2-methyl propane (0.26 mL) was dissolved in diethyl ether (5mL) and added dropwise, *via* an addition funnel, to a mixture of freshly ground magnesium turnings (0.054 g) and one crystal of iodine in diethyl ether (5mL). Reflux was initiated spontaneously and the solution was maintained at reflux, by use of an oil bath, for 30 minutes to form the Grignard reagent. The resulting solution was allowed to cool to room temperature. 3 β -Acetoxy-5 α -4,4,14-trimethyl-chol -8-ene-24-al **21** (0.200 g, 0.45 mM) dissolved in diethyl ether (10 ml) was added dropwise to the Grignard reagent, *via* an addition funnel, at 0 °C. The reaction mixture was allowed to warm to room temperature. The mixture was then stirred at room temperature for 1 h and at reflux for 6 h. The reaction mixture was removed from reflux and allowed to cool to room temperature. Ice cold saturated ammonium chloride solution (20 mL) was added and the ether layer was washed with saturated sodium carbonate (3×20 mL) and water (1×20 mL). The organic layer was dried over magnesium sulfate and concentrated under reduced pressure to yield the desired product **31** as a dark orange oil (0.144 g, 66%). $\nu_{max}(NaCl)/cm^{-1}$ 3441, 2953, 1734; 1H NMR spectrum shows grease is present in the sample. TMS standard was offset by 0.069 ppm and chemical shifts

are therefore corrected by calculation; δ_{H} (300 MHz, CDCl_3) 0.69 (3H, s, 18- CH_3), 0.78 – 2.15 [50H, m, containing 0.88 (6H, s, 28- CH_3 and 29- CH_3), 0.90 (3H, s, 21- CH_3), 1.00 (3H, s, 19- CH_3), 2.05 (3H, s, OAc, CH_3)], 3.52 – 3.72 (1H, m, C-24 O-H), 4.50 (1H, dd, J 11.3, 4.7, 3-CH). δ_{C} (75 MHz, CDCl_3) 15.8 (29- CH_3), 16.5 (18- CH_3), 18.1 (6- CH_2), 19.2 (19- CH_3), 21.0 (11- CH_2), 21.3 (21- CH_3), 22.0 (OAc, CH_3), 22.1 (30- CH_3), 23.5 (26- CH_3), 23.6 (27- CH_3), 24.17 (2- CH_2), 24.7 (25-CH), 26.4 (23- CH_2), 27.9 (28- CH_3), 28.2 (7- CH_2), 29.7 (16- CH_2), 30.8 (15- CH_2), 31.0 (12- CH_2), 35.3 (1- CH_2), 36.9 (4-quaternary C), 37.5 (20-CH), 37.8 (10-quaternary C), 44.5 (13-quaternary C), 46.9 (22- CH_2), 49.8 (14-quaternary C), 50.3 (5-CH or 17-CH), 50.5 (5-CH or 17-CH), 71.4 (24-CH R or S), 71.7 (24-CH R or S), 82.0 (3-CH), 134.3 (8-quaternary C or 9-quaternary C), 134.5 (8-quaternary C or 9-quaternary C), 171.0 (quaternary C, OAc, $\text{C}=\text{O}$).

3 β -Acetoxy-5 α -lanost-8-ene-24-ol- 26- methyl ester²⁰ **32**



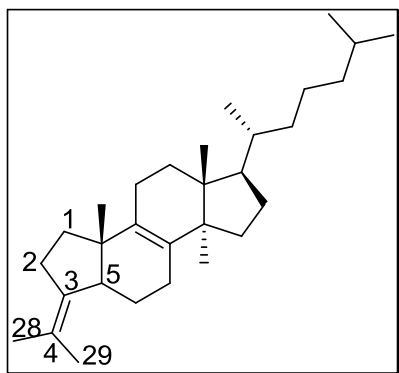
All glassware to be used for the Grignard reaction was dried overnight at 150 °C prior to use. The reaction was carried out in an anhydrous atmosphere, using a blanket of nitrogen at all stages of reagent addition. Glassware joints were greased to avoid air entering the reaction system. Methyl 3-bromo-2-

methylpropanoate (0.22 mL) was dissolved in diethyl ether (5mL) and added dropwise, *via* an addition funnel, to a mixture of freshly ground magnesium turnings (0.040 g) and one crystal of iodine in diethyl ether (5mL). Bubbling of the reaction solution initiated spontaneously. The solution was brought to reflux, by use of an oil bath, and maintained at reflux for 30 minutes to form the Grignard reagent. The resulting solution was allowed to cool to room temperature. 3 β -Acetoxy-5 α -4,4,14-trimethyl-chol-8-ene-24-al **21** (0.200 g, 0.45 mM) dissolved in diethyl ether (10 ml) was added dropwise to the Grignard reagent, *via* an addition funnel, at 0 °C. The reaction mixture was allowed to warm to room temperature. The mixture was then stirred at room temperature for 1 h and at reflux for 6 h. The reaction mixture was removed from reflux and allowed to cool to room temperature. Ice cold saturated ammonium

chloride solution (20 mL) was added and the ether layer was washed with saturated sodium carbonate (3×20 mL) and water (1×20 mL). The organic layer was dried over magnesium sulfate and concentrated under reduced pressure to yield the crude product as a yellow oil (0.178 g). Inspection of the ^1H NMR spectrum of the crude material showed that a mixture of compounds is present*. The starting aldehyde is still present due to the characteristic signal at 9.77 ppm. An unidentified alkene signal at 5.12 ppm is also observed in the spectrum. There is no evidence of the formation of the desired product **32**. There is an excess of bromide starting material in relation to the steroid component. Characteristic signals for methyl 3-bromo-2-methylpropanoate are: δ_{H} (300 MHz, CDCl_3) 1.23 (3H, d, J 7.0), 2.91 (1H, q, J 13.2, 6.7), 3.53 (2H, ddd, J 48.7, 10.0, 6.3) 3.73 (3H, s).

* As in the previous Grignard reaction description, TMS standard was offset by 0.070 ppm. Chemical shifts are therefore corrected by calculation.

3-Isopropyliden-14 α -methyl-A-nor-5 α -cholest-8-ene²¹ **35**

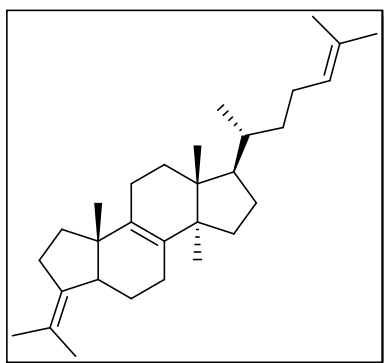


Phosphorous pentachloride (0.435 g, 2.09 mM) was added to a stirring solution of dihydrolanosterol **7** (0.435 g, 1.02 mM), which contained ~ 17% lanosterol **6** in hexane (44 mL). The reaction mixture was stirred at room temperature for 2 h after which time it was heated at reflux for 1 h. The reaction mixture was diluted with ether (50 mL), washed with water (4×50 mL), 5% sodium hydrogen carbonate (2×50 mL) and again with water (2×50 mL). The organic

layer was dried over magnesium sulfate and concentrated under reduced pressure to yield the crude product as a brown oil (0.382 g). ^1H NMR analysis of the crude material showed it contained the desired product, and a side product characterised by a free alkene hydrogen. Purification by column chromatography using hexane eluant led to isolation of the desired product **35** as white needles (0.162 g, 39%) and the side product **36** as yellow crystals (0.074 g, 9%). mp 143 – 147 °C (literature,²² 143 – 144 °C; δ_{H} (300 MHz, CDCl_3) 0.73 (3H, s, 18- CH_3), 0.80 (3H, s, 19- CH_3), 0.82 – 0.94 (12H, m, 30- CH_3 , 26- CH_3 , 27- CH_3 , 21- CH_3) 0.95 – 1.60 (22H, m), 1.61 – 1.82 (6H, m, 28- CH_3 , 29- CH_3) 1.83 – 2.41 (10H, m); δ_{C} (75 MHz, CDCl_3) 15.6 (18- CH_3), 18.8 (21- CH_3), 19.5 (19- CH_3), 19.7 (28- CH_3 or 29- CH_3), 22.57 (26- CH_3), 22.62 (CH_2), 22.8 (27- CH_3), 22.9 (28- CH_3 or 29- CH_3), 22.9 (11- CH_2), 24.1 (23- CH_2),

24.6 (CH₃), 25.8 (7-CH₂), 28.0 (25-CH), 28.3 (16-CH₂), 29.8 (6-CH₂), 30.6 (15-CH₂), 30.7 (12-CH₂), 34.5 (1-CH₂), 36.5 (20-CH), 36.6 (22-CH₂), 39.6 (24-CH₂), 44.4 (13-quaternary C), 44.9 (10-quaternary C), 49.3 (14-quaternary C), 50.3 (17-CH), 51.3 (5-CH), 121.6 (4-quaternary C), 133.6 (8-quaternary C or 9-quaternary C), 134.2 (8-quaternary C or 9-quaternary C), 135.2 (3-quaternary C).

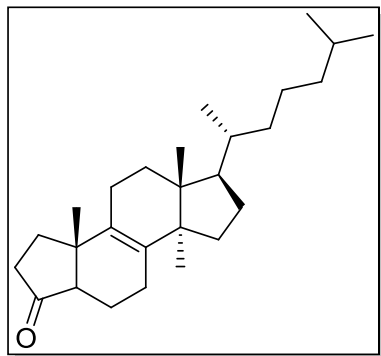
3-Isopropyliden-14 α -methyl-A-nor-5 α -cholest-8,24-diene^{21,22} **36**



Spectral details of the side product arising from the minor impurity in the starting material of the previous reaction: mp 135 – 138 °C (literature,²³ 136 – 140 °C); δ_H (300 MHz, CDCl₃) 0.65 – 2.45 [45H, m, containing 0.73 (3H, s, 18-CH₃), 0.80 (3H, s, 19-CH₃), 0.88 (3H, s, 30-CH₃), 0.93 (3H, d, *J* 6.3, 21-CH₃), 1.59 (3H, d, *J* 11.6, 27-CH₃), 1.71 (3H, d, *J* 15.1, 26-CH₃)], 5.12 (1H, t, *J* 7.1, 24-CH); δ_C (75 MHz,

CDCl₃) 15.6 (18-CH₃), 17.7 (27-CH₃), 18.7 (21-CH₃), 19.5 (19-CH₃), 19.7 (28-CH₃ or 29-CH₃), 22.6 (11-CH₂), 22.8 (27-CH₃), 22.9 (2-CH₂), 24.6 (30-CH₃), 24.9 (23-CH₂), 25.75 (26-CH₃), 25.82 (7-CH₂), 28.3 (16-CH₂), 29.8 (6-CH₂), 30.6 (15-CH₂), 30.7 (12-CH₂), 34.4 (1-CH₂), 36.3 (20-CH), 36.4 (22-CH₂), 44.4 (13-quaternary C), 44.9 (10-quaternary C), 49.3 (14-quaternary C), 50.3 (5-CH or 17-CH), 51.4 (5-CH or 17-CH), 121.6 (4-quaternary C), 125.3 (24-CH), 130.9 (25-quaternary C), 133.5 (8-quaternary C or 9-quaternary C), 134.2 (8-quaternary C or 9-quaternary C), 135.2 (3-quaternary C),

Attempted synthesis of 3-Isopropyliden-14 α -methyl-A-nor-5 α -cholest-8-ene²¹ **34**



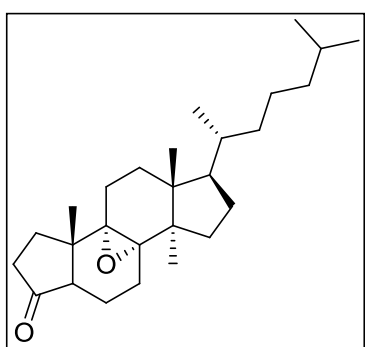
3-Isopropyliden-14- α -methyl-A-nor-5 α -lanost-8-ene **35**

(0.160 g, 0.42 mmol) was dissolved in hexane (10 mL) in a 100 ml Shlenck tube. The Shlenck tube inlet was connected via rubber tubing to an ozone generator. The outlet was connected to two dreschel bottles containing 350 mL and 150 mL 10% KI solution respectively. Oxygen was passed through the reaction apparatus for 5 minutes to ensure connections were well sealed. The ozone generator was switched on and ozone was passed

connections were well sealed. The ozone generator was switched on and ozone was passed

through this solution at $-78\text{ }^{\circ}\text{C}$ for 10 minutes. Excessive ozone was removed with a stream of oxygen for 5 minutes. Excess triphenylphosphine was added and the reaction mixture was stirred overnight to break down any residual ozonide. Celite was added directly to the reaction mixture in solvent. The solvent was removed *in vacuo*. Purification by column chromatography (hexane:ethyl acetate 90:10) led to the isolation of 14 α -methyl-8 α ,9 α -epoxy-A-nor-5 α -cholestan-3-one **37** (0.037g, 22%) as a clear oil.

14 α -methyl-8 α ,9 α -epoxy-A-nor-5 α -cholestan-3-one²¹ **37**

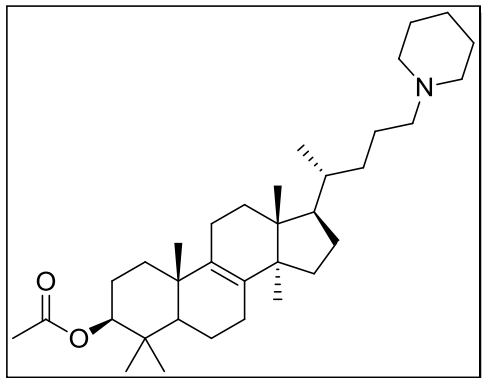


ν_{max} (film)/ cm^{-1} : 2954, 1742; δ_{H} (300 MHz, CDCl_3) 0.70 – 2.45 [43H, m, containing 0.80 (3H, s, 18- CH_3), 0.85 – 0.92 (12H, m, 26- CH_3 , 27- CH_3 , 21- CH_3 , 30- CH_3), 1.02 (3H, s, 19- CH_3), 2.29 (1H, dt, J 9.5, 1.5), 2.36 (1H, dt, J 7.1, 5.5), 2.68 (1H, dd, J 12.0, 3.8, 5 α -CH)]; δ_{C} (75 MHz, CDCl_3) 14.6 (6- CH_2), 16.2 (18- CH_3), 17.4 (19- CH_3), 19.0 (21- CH_3), 20.0 (30- CH_3), 21.3 (7- CH_2), 21.7 (11- CH_2), 22.5 (26- CH_3), 22.8 (27- CH_3), 24.0 (23- CH_2), 26.4 (12- CH_2), 28.0 (25-CH), 28.4 (16- CH_2), 28.9 (1- CH_2), 31.7 (15- CH_2), 34.58 (2- CH_2), 36.2 (20-CH), 36.3 (22- CH_2), 39.46 (24- CH_2), 43.76 and 43.98 (13-quaternary C and 10-quaternary C), 48.2 (5-CH), 48.6 (14-quaternary C), 51.9 (17-CH), 68.6 (8-quaternary C), 69.3 (9-quaternary C), 217.4 (3-quaternary C); m/z (ESI^+) 401 [$\text{M} + \text{H}$] $^+$, (42%), 383 (100), 279 (44); exact mass calculated for $\text{C}_{27}\text{H}_{45}\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 401.3420, found 401.3403.

3.3 Amine Derivatives of Lanosterol

Note: The reductive aminations described in this section were carried out on $\geq 95\%$ pure starting aldehydes.

3 β -Acetoxy-5 α -4,4,14-trimethyl-24-piperadino-chol-8-ene **41**

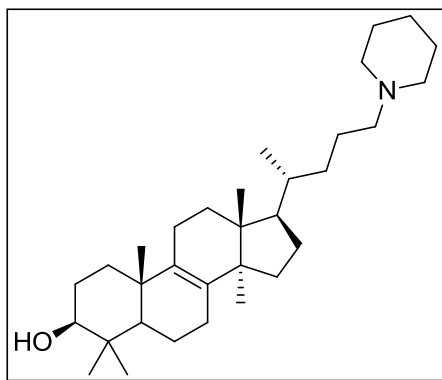


Sodium triacetoxyborohydride (0.067 g, 0.32 mM) was added to a solution of 3 β -acetoxy-5 α -4,4,14-trimethyl-chol-8-ene-24-al **21** (0.100 g, 0.23 mM) and piperidine (22 μ L, 0.23 mM) in 1,2-dichloroethane (10 mL). The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate solution (10 mL) was added. The reaction mixture was

extracted into ethyl acetate (10 mL) and washed with brine (2×10 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield the desired product **41** as an off white solid (0.110 g, 91%): mp 150 – 151 $^{\circ}$ C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 2940, 1735; δ_{H} (300 MHz, CDCl_3) 0.68 (3H, s, 18- CH_3), 0.80 – 0.95 (12H, m, containing 21- CH_3 , 28- CH_3 , 29- CH_3 , 30- CH_3), 1.00 (3H, s, 19- CH_3), 1.03 – 1.80 (24H, m), 1.82 – 2.15 [8H, m, containing 2.05 (3H, s, OAc, CH_3)], 2.25 – 2.30 (2H, m, 24- CH_2), 2.41 (4H, br s, piperidine $\text{CH}_2 \times 2$), 4.50 (1H, dd, J 11.5, 4.5, 3-CH); δ_{C} (75 MHz, CDCl_3)²⁴ 15.7 (18- CH_3), 16.5 (29- CH_3), 18.1 (6- CH_2), 18.7 (21- CH_3), 19.2 (19- CH_3), 21.0 (11- CH_2), 21.3 (OAc, CH_3), 23.4 (23- CH_2), 24.17 (2- CH_2), 24.24 (30- CH_3), 24.4 (piperidine CH_2), 25.8 (piperidine $\text{CH}_2 \times 2$), 26.4 (7- CH_2), 27.9 (28- CH_3), 28.2 (16- CH_2), 30.8 (15- CH_2), 31.0 (12- CH_2), 34.1 (22- CH_2), 35.3 (1- CH_2), 36.4 (20-CH), 36.9 (10-quaternary C), 37.8 (4-quaternary C), 44.5 (13-quaternary C), 49.8 (14-quaternary C), 50.3 (5-CH or 17-CH), 50.5 (5-CH or 17-CH), 54.5 (piperidine N- $\text{CH}_2 \times 2$), 59.9 (24- CH_2), 80.9 (3-CH), 134.3 (9-quaternary C), 134.5 (8-quaternary C), 171.0 (quaternary C, OAc, C=O); m/z (ESI^+) 512 [$\text{M} + \text{H}$]⁺; exact mass calculated for $\text{C}_{34}\text{H}_{58}\text{NO}_2$ [$\text{M} + \text{H}$]⁺ 512.4468, found 512.4476.

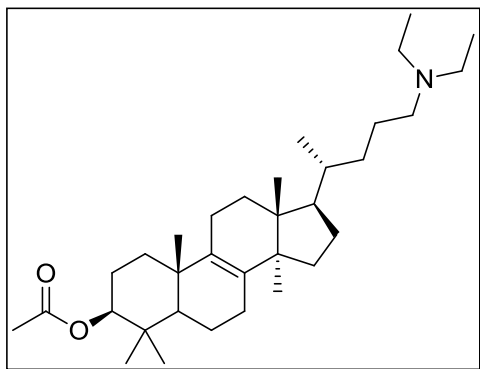
Reaction was also carried out using piperidine (4.2 eq), NaBH_3CN (3.6 eq), ZnCl_2 (1.8 eq) in MeOH at room temperature for 16 h, resulting in 84% yield.

For reductive aminations in this section: Procedure as per Abdel-Magid reference.²⁵

3 β -Hydroxy-5 α -4,4,14-trimethyl-24-piperadino-chol-8-ene 42

Sodium triacetoxyborohydride (0.074 g, 0.35 mM) was added to a solution of 3 β -hydroxy-5 α -4,4,14-trimethyl-chol-8-ene-24-al **25** (0.100 g, 0.25 mM) and piperidine (25 μ L, 0.25 mM) in 1,2-dichloroethane (10 mL). The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate solution (10 mL) was added. The reaction mixture was extracted into ethyl acetate (10 mL) and washed with brine (2×10 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield the desired product **42** as a white solid (0.100 g, 85%) mp 119 – 121 $^{\circ}$ C; ν_{max} (KBr)/ cm^{-1} 3432, 2941; δ_{H} (300 MHz, CDCl_3) 0.69 (3H, s, 18- CH_3), 0.75 – 1.80 [40H, m, containing 0.81 (3H, s, 29- CH_3), 0.87 (3H, s, 30- CH_3), 0.90 (3H, d, J 6.2, 21- CH_3), 0.98 (3H, s, 19- CH_3), 1.00 (3H, s, 28- CH_3)], 1.81 – 2.13 (6H, m), 2.20 – 2.30 (2H, m, 24- CH_2), 2.31 – 2.52 (4H, m, piperidine $\text{CH}_2 \times 2$), 3.23 (1H, dd, J 4.5, 11.5, 3-CH); δ_{C} (75 MHz, CDCl_3) 15.4 (29- CH_3), 15.7 (18- CH_3), 18.3 (6- CH_2), 18.8 (21- CH_3), 19.1 (19- CH_3), 21.0 (11- CH_2), 23.4 (23- CH_2), 24.3 (30- CH_3), 24.4 (piperidine CH_2), 25.7 (piperidine $\text{CH}_2 \times 2$), 26.5 (7- CH_2), 27.9 (2- CH_2), 28.0 (28- CH_3), 28.2 (16- CH_2), 30.8 (15- CH_2), 31.0 (12- CH_2), 34.1 (22- CH_2), 35.6 (1- CH_2), 36.3 (20-CH), 37.0 (10-quaternary C), 38.9 (4-quaternary C), 44.5 (13-quaternary C), 49.8 (14-quaternary C), 50.3 (5-CH), 50.4 (17-CH), 54.6 (piperidine N- $\text{CH}_2 \times 2$), 60.0 (24- CH_2), 78.9 (3-CH), 134.37 (8-quaternary C or 9-quaternary C), 134.42 (8-quaternary C or 9-quaternary C). The following peaks were distinguishable for the minor dihydrolanosterol component **7** at a level <4%: 22.5 (26- CH_3), 22.8 (27- CH_3); m/z (ESI^+) 470 [$\text{M} + \text{H}$] $^+$ (100%); exact mass calculated for $\text{C}_{33}\text{H}_{56}\text{NO}$ [$\text{M} + \text{H}$] $^+$ 470.4362, found 470.4362.

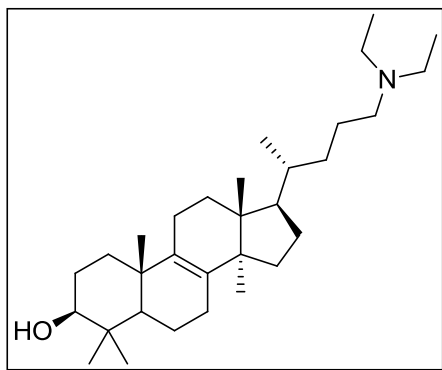
Assignments for piperidine derivatives are based on Lavrado paper.²⁶

3 β -Acetoxy-5 α -4,4,14-trimethyl-24-diethylamino-chole-8-ene 43

Sodium triacetoxyborohydride (0.067 g, 0.32 mM) was added to a solution of 3 β -acetoxy-5 α -4,4,14-trimethyl-chole-8-ene-24-al **21** (0.100 g, 0.23 mM) and diethylamine (23 μ L, 0.23 mM) in 1,2-dichloroethane (10 mL). The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate solution

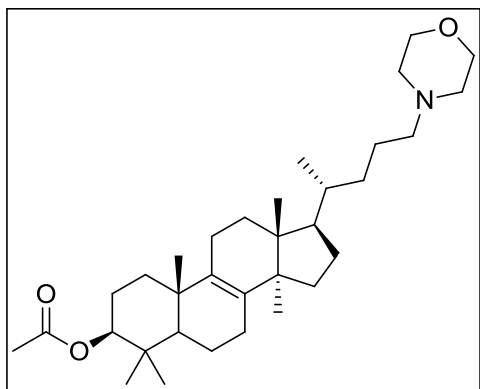
(10 mL) was added. The reaction mixture was extracted into ethyl acetate (10 mL) and washed with brine (2×10 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield the desired product **43** as a white solid (0.073 g, 65%) mp 87 – 89 $^{\circ}$ C; ν_{max} (KBr)/ cm^{-1} 2953, 1735, 1714; δ_{H} (300 MHz, CDCl_3) 0.68 (3H, s, 18- CH_3), 0.78 – 0.95 (12H, m, containing 21- CH_3 , 28- CH_3 , 29- CH_3 , 30- CH_3), 0.96 – 1.10 [9H, m, containing 1.00 (3H, s, 19- CH_3) and 1.06 (6H, t, J 7.2, diethylamine $\text{CH}_3 \times 2$)], 1.12 – 1.80 (12H, m), 1.81 – 2.15 (8H, m, containing 2.05 (3H, s, OAc, CH_3), 2.32 – 2.51 (2H, m, 24- CH_2), 2.52 – 2.67 (4H, m, diethylamine $\text{CH}_2 \times 2$), 4.50 (1H, dd, J 11.5, 4.5, 3-CH); δ_{C} (300 MHz, CDCl_3) 11.2 (diethylamine $\text{CH}_3 \times 2$), 15.8 (18- CH_3), 16.5 (29- CH_3), 18.1 (6- CH_2), 18.7 (21- CH_3), 19.2 (19- CH_3), 21.0 (11- CH_2), 21.3 (OAc, CH_3), 23.1 (23- CH_2), 24.16 (2- CH_2), 24.23 (30- CH_3), 26.4 (7- CH_2), 27.9 (28- CH_3), 28.2 (16- CH_2), 30.8 (15- CH_2), 31.0 (12- CH_2), 34.0 (22- CH_2), 35.3 (1- CH_2), 36.4 (20-CH), 36.9 (10-quaternary C), 37.8 (4-quaternary C), 44.5 (13-quaternary C), 46.6 (diethylamine N- $\text{CH}_2 \times 2$), 49.8 (14-quaternary C), 50.4 (5-CH or 17-CH), 50.5 (5-CH or 17-CH), 53.2 (24- CH_2), 80.9 (3-CH), 134.3 (8-quaternary C or 9-quaternary C), 134.5 (8-quaternary C or 9-quaternary C), 171.0 (quaternary C, OAc, C=O); m/z (ESI^+) 498 [$\text{M} - \text{H}]^+$ (18%) 316 (100); exact mass calculated for $\text{C}_{33}\text{H}_{58}\text{NO}_2$ [$\text{M} + \text{H}]^+$ 500.4468, found 500.4465.

The following peaks were distinguishable for the minor dihydrolanosterol acetate component at a level <4%: 22.5 (26- CH_3), 22.8 (27- CH_3), 27.9 (25-CH). Although no aldehyde signal can be seen at ~ 9 ppm in the ^1H NMR spectrum, a trace amount of aldehyde is present in the product due to a very minor signal at 206.5 ppm in the ^{13}C NMR spectrum.

3 β -Hydroxy-5 α -4,4,14-trimethyl-24-diethylamino-chole-8-ene 44

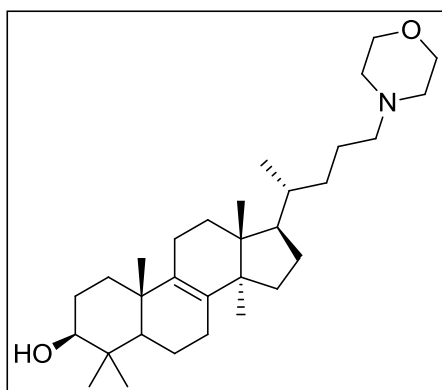
Sodium triacetoxyborohydride (0.074 g, 0.35 mM) was added to a solution of 3 β -hydroxy-5 α -4,4,14-trimethyl-chole-8-ene-24-al **25** (0.100 g, 0.25 mM) and diethylamine (26 μ L, 0.25 mM) in 1,2-dichloroethane (10 mL). The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate solution (10 mL) was added. The reaction mixture was extracted into ethyl acetate (10 mL) and washed with brine (2 \times 10 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield the desired product **44** as a white solid (0.086 g, 75%): mp 135 – 137 $^{\circ}$ C; ν_{max} (KBr)/ cm^{-1} 3432, 2944; δ_{H} (300 MHz, CDCl_3) 0.69 (3H, s, 18- CH_3), 0.74 – 1.80 [43H, m, containing 0.81 (3H, s, 29- CH_3), 0.87 (3H, s, 30- CH_3), 0.91 (3H, d, J 6.3, 21- CH_3), 0.98 (3H, s, 19- CH_3), 1.00 (3H, s, 28- CH_3), 1.07 (6H, t, J 7.2, diethylamine $\text{CH}_3 \times 2$)], 1.81 – 2.30 (4H, m), 2.38 – 2.50 (2H, m, 24- CH_2), 2.54 – 2.64 (4H, m, diethylamine $\text{CH}_2 \times 2$), 3.23 (1H, dd, J 11.5, 4.5, 3-CH); δ_{C} (75 MHz, CDCl_3) 11.2 (diethylamine $\text{CH}_3 \times 2$), 15.4 (29- CH_3), 15.8 (18- CH_3), 18.3 (6- CH_2), 18.7 (21- CH_3), 19.1 (19- CH_3), 21.0 (11- CH_2), 23.2 (23- CH_2), 24.3 (30- CH_3), 26.5 (7- CH_2), 27.9 (2- CH_2), 28.0 (28- CH_3), 28.2 (16- CH_2), 30.8 (15- CH_2), 31.0 (12- CH_2), 34.1 (22- CH_2), 35.6 (1- CH_2), 36.4 (20-CH), 37.0 (10-quaternary C), 38.9 (4-quaternary C), 44.5 (13-quaternary C), 46.8 (diethylamine N- $\text{CH}_2 \times 2$), 49.8 (14-quaternary C), 50.38 (5-CH or 17-CH), 50.41 (5-CH or 17-CH), 53.3 (24- CH_2), 79.0 (3-CH), 134.4 (8-quaternary C or 9-quaternary C), 134.4 (8-quaternary C or 9-quaternary C). The following peaks were distinguishable for the minor dihydrolanosterol component at a level <4%: 22.6 (26- CH_3), 22.9 (27- CH_3), 29.7 (25-CH); m/z (ESI $^{+}$) 458 [$\text{M} + \text{H}$] $^{+}$ (100%); exact mass calculated for $\text{C}_{31}\text{H}_{56}\text{NO}$ [$\text{M} + \text{H}$] $^{+}$ 458.4362, found 458.4366.

Assignments for diethylamine derivatives are based on Lavrado paper²⁶ and Csuk paper²⁷

3 β -Acetoxy-5 α -4,4,14-trimethyl-24-morpholino-chol-8-ene 45

Sodium triacetoxyborohydride (0.100 g, 0.47 mM) was added to a solution of 3 β -acetoxy-5 α -4,4,14-trimethyl-chol-8-ene-24-al **21** (0.150 g, 0.34 mM) and morpholine (30 μ L, 0.34 mM) in 1,2-dichloroethane (40 mL). The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate solution (20 mL) was added. The reaction mixture was

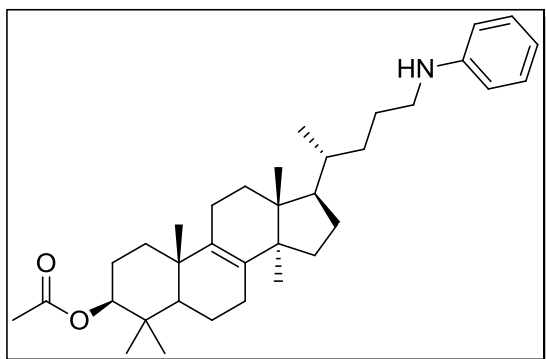
extracted into ethyl acetate (10 mL) and washed with brine (2 \times 40 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield the desired product **45** as an off white solid (0.094 g, 54%): mp 135 – 137 $^{\circ}$ C; Found C, 77.53; H, 10.60. C₃₃H₅₅NO₃ requires C, 77.14; H, 10.79%; ν_{\max} (KBr)/cm⁻¹ 2949, 1737; δ_{H} (300 MHz, CDCl₃) 0.69 (3H, s, 18-CH₃), 0.80 – 0.94 [12H, m, containing 21-CH₃, 28-CH₃, 29-CH₃, 30-CH₃], 1.00 (3H, s, 19-CH₃), 1.03 – 1.80 (18H, m), 1.82 – 2.10 [8H, m, containing 2.05 (3H, s, OAc, CH₃)], 2.29 (2H, t, *J* 7.1, 24-CH₂), 2.44 (4H, br s, morpholine N-CH₂ \times 2), 3.72 (4H, t, *J* 4.7, morpholine O-CH₂ \times 2), 4.50 (1H, dd, *J* 11.5, 4.5, 3-CH); δ_{C} (75 MHz, CDCl₃) 15.8 (18-CH₃), 16.5 (29-CH₃), 18.1 (6-CH₂), 18.7 (21-CH₃), 19.2 (19-CH₃), 21.0 (11-CH₂), 21.3 (OAc, CH₃), 23.2 (23-CH₂), 24.17 (2-CH₂), 24.24 (30-CH₃), 26.4 (7-CH₂), 27.9 (28-CH₃), 28.2 (16-CH₂), 30.8 (15-CH₂), 31.0 (12-CH₂), 33.9 (22-CH₂), 35.3 (1-CH₂), 36.3 (20-CH), 36.9 (10-quaternary C), 37.8 (4-quaternary C), 44.5 (13-quaternary C), 49.8 (14-quaternary C), 50.3 (5-CH or 17-CH), 50.5 (5-CH or 17-CH), 53.8 (morpholine N-CH₂ \times 2), 59.7 (24-CH₂), 67.0 (morpholine O-CH₂ \times 2), 80.9 (3-CH), 134.3 (9-quaternary C), 134.5 (8-quaternary C), 171.0 (quaternary C, OAc, C=O); *m/z* (ESI⁺) 514 [M + H]⁺ (100%); exact mass calculated for C₃₃H₅₆NO₃ [M + H]⁺ 514.4260, found 514.4257. The following peaks were distinguishable for the minor dihydrolanosterol acetate component at a level <4%: 22.5 (26-CH₃), 22.8 (27-CH₃), 28.0 (25-CH).

3 β -Acetoxy-5 α -4,4,14-trimethyl-24-morpholino-chol-8-ene 46

Sodium triacetoxyborohydride (0.148 g, 0.70 mM) was added to a solution of 3 β -hydroxy-5 α -4,4,14-trimethylchol-8-ene-24-al **25** (0.200 g, 0.50 mM) and morpholine (39 μ L, 0.50 mM) in 1,2-dichloroethane (40 mL). The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate solution (20 mL) was added. The reaction mixture was extracted into ethyl acetate (10 mL) and

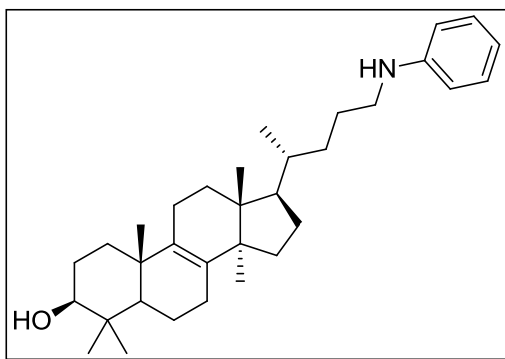
washed with brine (2×40 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure²⁵ to yield the desired product **46** as an off white solid (0.204 g, 87%): mp 132 – 134 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3422, 2955; δ_{H} (300 MHz, CDCl_3) 0.69 (3H, s, 18- CH_3), 0.75 – 1.81 [34H, m, containing 0.81 (3H, s, 29- CH_3), 0.87 (3H, s, 30- CH_3), 0.91 (3H, d, J 6.2, 21- CH_3), 0.98 (3H, s, 19- CH_3), 1.00 (3H, s, 28- CH_3)], 1.83 – 2.16 (5H, m), 2.29 (2H, t, J 6.8, 24- CH_2), 2.44 (4H, br s, morpholine N- $\text{CH}_2 \times 2$), 3.23 (1H, dd, J 11.5, 4.5, 3-CH), 3.72 (4H, t, J 4.7, morpholine O- $\text{CH}_2 \times 2$); δ_{C} (75 MHz, CDCl_3) 15.4 (29- CH_3), 15.7 (18- CH_3), 18.3 (6- CH_2), 18.7 (21- CH_3), 19.1 (19- CH_3), 21.0 (11- CH_2), 23.2 (23- CH_2), 24.2 (30- CH_3), 26.5 (7- CH_2), 27.8 (2- CH_2), 28.0 (28- CH_3), 28.2 (16- CH_2), 30.8 (15- CH_2), 31.0 (12- CH_2), 33.9 (22- CH_2), 35.6 (1- CH_2), 36.3 (20-CH), 37.0 (10-quaternary C), 38.9 (4-quaternary C), 44.5 (13-quaternary C), 49.8 (14-quaternary C), 50.3 (5-CH or 17-CH), 50.4 (5-CH or 17-CH), 53.8 (morpholine N- $\text{CH}_2 \times 2$), 59.7 (N- CH_2), 67.0 (morpholine O- $\text{CH}_2 \times 2$), 79.0 (3-CH), 134.3 (9-quaternary C), 134.5 (8-quaternary C); m/z (ESI⁺) 472 [$\text{M} + \text{H}$]⁺ (100%); exact mass calculated for $\text{C}_{31}\text{H}_{54}\text{NO}_2$ 472.4155, found 472.4153.

Assignments for morpholine derivatives are based on Taniguchi paper²⁸

3 β -Acetoxy-5 α -4,4,14-trimethyl-24-anilino-chole-8-ene 47

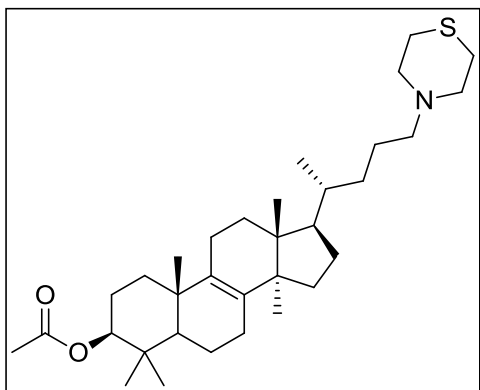
Sodium triacetoxyborohydride (0.067 g, 0.32 mM) was added to a solution of 3 β -acetoxy-5 α -4,4,14-trimethyl-chole-8-ene-24-al **21** (0.100 g, 0.23 mM) and aniline (21 μ L, 0.23 mM) in 1,2-dichloroethane (35 mL). The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium

bicarbonate solution (20 mL) was added. The reaction mixture was extracted into ethyl acetate (10 mL) and washed with brine (2 \times 40 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield the desired product **47** as an off white oily solid (0.160 g, 94%): Found C, 80.11; H, 10.42. C₃₅H₅₃NO₂ requires C, 80.87; H, 10.28%; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 2949, 1733; δ_{H} (300 MHz, CDCl₃) 0.69 (3H, s, 18-CH₃), 0.76 – 1.03 [16H, m, containing 21-CH₃, 28-CH₃, 29-CH₃, 30-CH₃ and 1.00 (3H, s, 19-CH₃)], 1.04 – 1.83 (18H, m), 1.84 – 2.15 [8H, m, containing 2.05 (3H, s, OAc, CH₃)], 3.01 – 3.13 (2H, m, 24-CH₂), 4.50 (1H, dd, *J* 11.6, 4.5, 3-CH), 6.59 (2H, d, *J* 7.7, Ar-CH \times 2), 6.67 (1H, t, *J* 7.3, *p*-Ar CH), 7.14 – 7.18 (2H, m, Ar-CH); δ_{C} (75 MHz, CDCl₃) 15.8 (18-CH₃), 16.6 (29-CH₃), 18.1 (6-CH₂), 18.7 (21-CH₃), 19.2 (19-CH₃), 21.0 (11-CH₂), 21.3 (OAc, CH₃), 24.2 (2-CH₂), 24.3 (30-CH₃), 26.4 (7-CH₂), 27.9 (28-CH₃), 28.2 (16-CH₂), 30.8 (15-CH₂), 31.0 (12-CH₂), 33.7 (22-CH₂), 35.3 (1-CH₂), 36.3 (20-CH), 36.9 (10-quaternary C), 37.8 (4-quaternary C), 44.5 (13-quaternary C), 44.6 (24-CH₂), 49.8 (14-quaternary C), 50.4 (5-CH or 17-CH), 50.5 (5-CH or 17-CH), 80.9 (3-CH), 112.7 (Ar-CH \times 2), 117.1 (Ar-CH), 129.2 (Ar-CH \times 2), 134.3 (8-quaternary C or 9-quaternary C), 134.5 (8-quaternary C or 9-quaternary C), 148.6 (Ar-quaternary C), 171.0 (OAc, C=O, quaternary C). The following peaks were distinguishable for the minor dihydrolanosterol acetate component at a level <4%: 22.6 (26-CH₃), 22.9 (27-CH₃), 29.7 (25-CH); *m/z* (ESI⁺) 520 [M + H]⁺ (12%) 102 (100); exact mass calculated for C₃₅H₅₄NO₂ [M + H]⁺ 520.4155, found 520.4138.

3 β -Hydroxy-5 α -4,4,14-trimethyl-24-anilino-chol-8-ene 48

Sodium triacetoxyborohydride (0.015 g, 0.07 mM) was added to a solution of 3 β -hydroxy-5 α -4,4,14-trimethyl-chol-8-ene-24-al **25** (0.020 g, 0.05 mM) and aniline (4.5 μ L, 0.05 mM) in 1,2-dichloroethane (15 mL). The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate

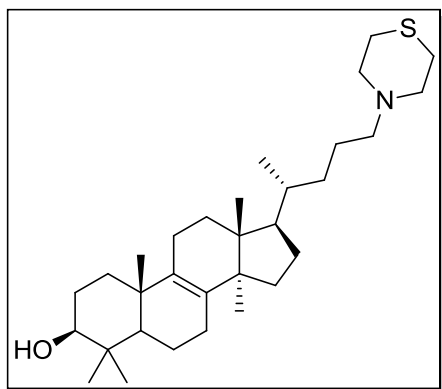
solution (10 mL) was added. The reaction mixture was extracted into ethyl acetate (10 mL) and washed with brine (2 \times 20 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield the desired product **48** as an off white oily solid (0.024 g, 93%): $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3324, 2951; δ_{H} (300 MHz, CDCl_3) 0.60 – 2.20 [43H, m, containing 0.69 (3H, s, 18- CH_3), 0.81 (3H, s, 29- CH_3), 0.88 (3H, s, 30- CH_3), 0.92 (3H, d, J 6.1, 21- CH_3), 0.98 (3H, s, 19- CH_3), 1.00 (3H, s, 28- CH_3)], 2.98 – 3.15 (2H, m, 24- CH_2), 3.23 (1H, dd, J 11.5, 4.5, 3-CH), 6.60 (2H, d, J 7.7, Ar-CH \times 2), 6.68 (1H, t, J 7.2, p -Ar CH), 7.14 – 7.18 (2H, m, Ar-CH \times 2); δ_{C} (75 MHz, CDCl_3) 15.4 (29- CH_3), 15.8 (18- CH_3), 18.3 (6- CH_2), 18.7 (21- CH_3), 19.1 (19- CH_3), 21.0 (11- CH_2), 24.3 (30- CH_3), 26.4 (CH_2), 26.5 (7- CH_2), 27.9 (2- CH_2), 28.0 (28- CH_3), 28.2 (16- CH_2), 29.7 (CH_2), 30.8 (15- CH_2), 31.0 (12- CH_2), 33.6 (22- CH_2), 35.6 (1- CH_2), 36.3 (20-CH), 37.0 (10-quaternary C), 44.5 (13-quaternary C), 44.6 (24- CH_2), 49.8 (14-quaternary C), 50.39 (5-CH or 17-CH), 50.42 (5-CH or 17-CH), 79.0 (3-CH), 112.7 (Ar-CH \times 2), 117.1 (Ar-CH), 129.2 (Ar-CH \times 2), 134.4 (8-quaternary C or 9-quaternary C), 134.5 (8-quaternary C or 9-quaternary C); exact mass calculated for $\text{C}_{33}\text{H}_{52}\text{NO}$ $[\text{M} + \text{H}]^+$ 478.4049, found 478.4041.

3 β -Acetoxy-5 α -4,4,14-trimethyl-24-thiomorpholino-chol-8-ene 49

Sodium triacetoxyborohydride (0.080 g, 0.38 mM) was added to a solution of 3 β -acetoxy-5 α -4,4,14-trimethyl-chol-8-ene-24-al **21** (0.120 g, 0.27 mM) and thiomorpholine (27 μ L, 0.27 mM) in 1,2-dichloroethane (40 mL). The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate solution

(20 mL) was added. The reaction mixture was extracted into ethyl acetate (10 mL) and washed with brine (2×40 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield the desired product **49** as an orange solid (0.142 g, 71%): mp 137 – 139 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 2949, 1735, 1715; δ_{H} (300 MHz, CDCl_3) 0.68 (3H, s, 18- CH_3), 0.80 – 0.92 [12H, m, containing 21- CH_3 , 28- CH_3 , 29- CH_3 , 30- CH_3], 1.00 (3H, s, 19- CH_3), 1.05 – 1.80 (20H, m), 1.81 – 2.10 [8H, m, containing (2.05, 3H, s, OAc, CH_3)], 2.20 – 2.40 (2H, m, 24- CH_2), 2.60 – 2.80 (8H, m, thiomorpholine $\text{CH}_2 \times 4$), 4.45 (1H, dd, J 11.5, 4.5, 3-CH); δ_{C} (75 MHz, CDCl_3) 15.8 (18- CH_3), 16.5 (29- CH_3), 18.1 (6- CH_2), 18.7 (21- CH_3), 19.2 (19- CH_3), 21.0 (11- CH_2), 21.3 (OAc, CH_3), 23.2 (23- CH_2), 24.2 (2- CH_2), 24.3 (30- CH_3), 26.4 (7- CH_2), 27.9 (28- CH_3), 28.0 (thiomorpholine S- $\text{CH}_2 \times 2$), 28.2 (16- CH_2), 30.8 (15- CH_2), 31.0 (12- CH_2), 33.9 (22- CH_2), 35.3 (1- CH_2), 36.3 (20-CH), 36.9 (10-quaternary C), 37.8 (4-quaternary C), 44.5 (13-quaternary C), 49.8 (14-quaternary C), 50.3 (5-CH or 17-CH), 50.5 (5-CH or 17-CH), 55.1 (thiomorpholine N- $\text{CH}_2 \times 2$), 59.9 (24- CH_2), 80.9 (3-CH), 134.1 (8-quaternary C or 9-quaternary C), 134.3 (8-quaternary C or 9-quaternary C), 171.0 (quaternary C, OAc, C=O); m/z (ESI^+) 530 [$\text{M} + \text{H}$] $^+$ (12%), 326 (100); exact mass calculated for $\text{C}_{33}\text{H}_{55}\text{NO}_2\text{S}$ [$\text{M} + \text{H}$] $^+$ 530.4032, found 530.4044.

3 β -Acetoxy-5 α -4,4,14-trimethyl-24-thiomorpholino-chol-8-ene **50**

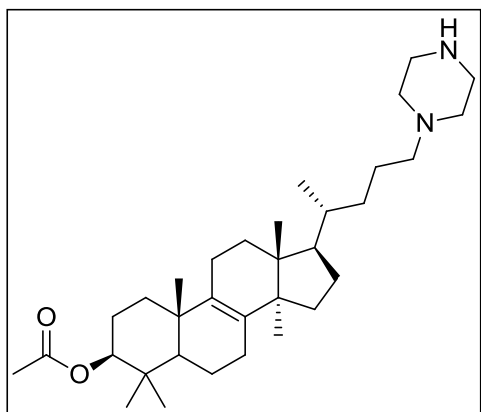


Sodium triacetoxyborohydride (0.059 g, 0.28 mM) was added to a solution of 3 β -hydroxy-5 α -4,4,14-trimethylchol-8-ene-24-al **25** (0.080 g, 0.20 mM) and thiomorpholine (20 μL , 0.20 mM) in 1,2-dichloroethane (40 mL). The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate solution (20 mL) was added. The reaction mixture was extracted into ethyl

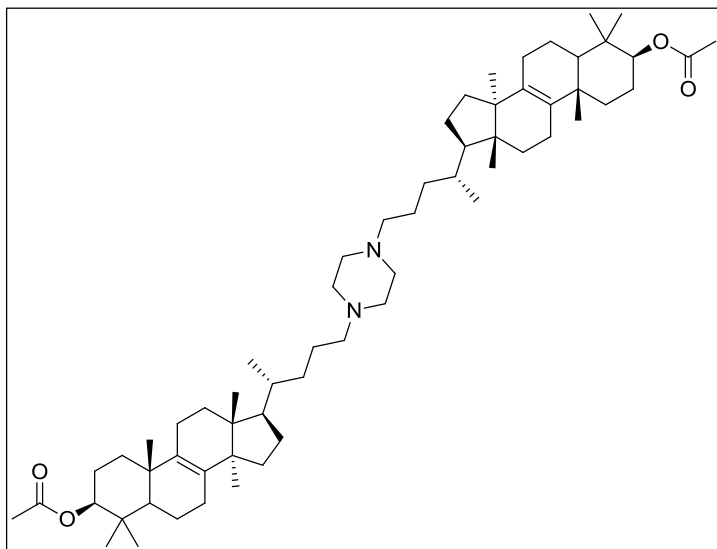
acetate (10 mL) and washed with brine (2×40 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield the desired product **50** as an orange solid (0.094 g, 96%), mp 131 – 134 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3316, 2939; δ_{H} (300 MHz, CDCl_3) 0.68 (3H, s, 18- CH_3), 0.75 – 1.10 [16H, m, containing 0.81 (3H, s, 29- CH_3), 0.87 (3H, s, 30- CH_3), 0.90 (3H, d, J 6.3, 21- CH_3), 0.98 (3H, s, 19- CH_3), 1.00 (3H, s, 28- CH_3)], 1.11 – 1.79 (18H, m), 1.81 – 2.13 (5H, m), 2.21 – 2.40 (2H, m, 24- CH_2), 2.57 – 2.80

(8H, m, thiomorpholine $\text{CH}_2 \times 4$), 3.23 (1H, dd, J 11.5, 4.6, 3-CH); δ_{C} (75 MHz, CDCl_3) 15.4 (29- CH_3), 15.7 (18- CH_3), 18.3 (6- CH_2), 18.8 (21- CH_3), 19.1 (19- CH_3), 21.0 (11- CH_2), 23.1 (23- CH_2), 24.3 (30- CH_3), 26.5 (7- CH_2), 27.9 (2- CH_2), 28.0 (thiomorpholine S- $\text{CH}_2 \times 2$), 28.2 (16- CH_2), 30.8 (15- CH_2), 31.0 (12- CH_2), 33.9 (22- CH_2), 35.6 (1- CH_2), 36.3 (20-CH), 37.0 (10-quaternary C), 38.9 (4-quaternary C), 44.5 (13-quaternary C), 49.8 (14-quaternary C), 50.3 (5-CH or 17-CH), 50.4 (5-CH or 17-CH), 55.1 (thiomorpholine N- $\text{CH}_2 \times 2$), 60.0 (24- CH_2), 78.9 (3-CH), 134.35 (8-quaternary C or 9-quaternary C), 134.44 (8-quaternary C or 9-quaternary C); m/z (ESI⁺) 488 $[\text{M} + \text{H}]^+$ (100%); exact mass calculated for $\text{C}_{31}\text{H}_{54}\text{NOS}$ $[\text{M} + \text{H}]^+$ 488.3926, found 488.3912.

Attempted synthesis of 3 β -acetoxy-5 α -4,4,14-trimethyl-24-piperazino-chol-8-ene **55**

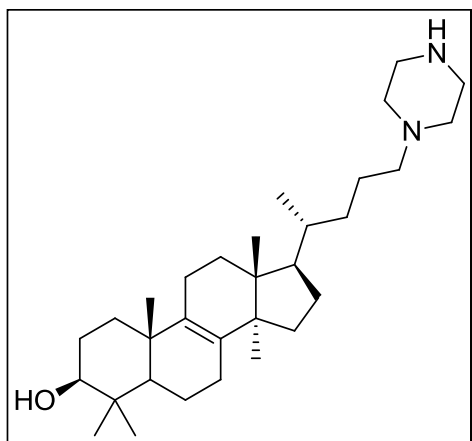


Sodium triacetoxyborohydride (0.134 g, 0.73 mM) was added to a solution of 3 β -acetoxy-5 α -4,4,14-trimethyl-chol-8-ene-24-al **21** (0.200 g, 0.45 mM) and piperazine (0.038 g, 0.45 mM) in 1,2-dichloroethane (40 mL). The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate solution (20 mL) was added. The reaction mixture was extracted into ethyl acetate (20 mL) and washed with brine (2×40 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield 3 β -acetoxy-5 α -4,4,14-trimethyl-24-piperazino-chol-8-ene dimer **56** as a white solid (0.120 g, 52%), with no evidence of formation of the desired product **55**.

3 β -Acetoxy-5 α -4,4,14-trimethyl-24-piperazino-chol-8-ene dimer 56

mp 200 – 202 °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 2945, 1734, 1287; δ_{H} (300 MHz, CDCl_3)²⁹ 0.69 (6H, s, 18-CH₃ × 2), 0.79 – 0.93 (24H, m, 21-CH₃ × 2, 28-CH₃ × 2, 29-CH₃ × 2, 30-CH₃ × 2), 0.94 – 1.10 [8H, m, containing 1.00 (6H, s, 19-CH₃ × 2)], 1.11 – 1.80 (40H, m), 1.81 – 2.15 [16H, m containing 2.05 (3H, s, OAc, CH₃ × 2)], 2.19 – 2.32 (4H, m, 24-CH₂), 2.33 – 2.70

(8H, m, piperazine CH₂ × 4), 4.50 (2H, dd, J 11.2, 4.7, 3-CH × 2); δ_{C} (75 MHz, CDCl_3) 15.7 (18-CH₃), 16.5 (29-CH₃), 18.1 (6-CH₂), 18.8 (21-CH₃), 19.2 (19-CH₃), 21.0 (11-CH₂), 21.4 (OAc, CH₃), 23.6 (23-CH₂), 24.2 (2-CH₂), 24.3 (30-CH₃), 26.4 (7-CH₂), 27.9 (28-CH₃), 28.2 (16-CH₂), 30.8 (15-CH₂), 30.9 (12-CH₂), 34.0 (22-CH₂), 35.2 (1-CH₂), 36.4 (20-CH), 36.9 (10-quaternary C), 37.8 (4-quaternary C), 44.4 (13-quaternary C), 50.2 (5-CH or 17-CH), 50.5 (5-CH or 17-CH), 53.3 (piperazine CH₂ × 4), 59.4 (24-CH₂), 80.9 (3-CH), 134.2 (8-quaternary C or 9-quaternary C), 134.4 (8-quaternary C or 9-quaternary C), 170.0 (quaternary C, OAc, C=O); exact mass calculated for C₆₂H₁₀₃N₂O₄ [M + H]⁺ 939.7918, found 939.7879.

3 β -Hydroxy-5 α -4,4,14-trimethyl-24-piperazino-chol-8-ene 57

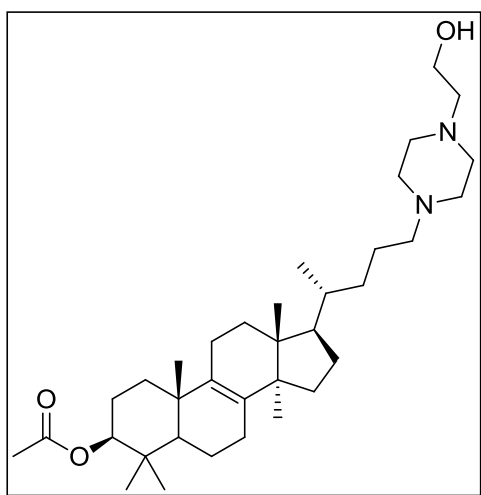
Sodium triacetoxyborohydride (0.074 g, 0.35 mM) was added to a solution of 3 β -hydroxy-5 α -4,4,14-trimethyl-chol-8-ene-24-al **25** (0.100 g, 0.25 mM) and piperazine (0.022 g, 0.25 mM) in 1,2-dichloroethane (30 mL). The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate solution (20 mL) was added. The reaction mixture was extracted into ethyl acetate (20 mL) and washed with brine (2 × 20 mL).

The organic extracts were then dried over magnesium sulfate and concentrated under reduced

pressure to yield the desired product **57** as an orange solidifying oil (0.089 g, 76%): $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3431, 2944; δ_{H} (300 MHz, CDCl_3) 0.69 (3H, s, 18- CH_3), 0.72 – 1.80 [35H, m, containing 0.81 (3H, s, 29- CH_3), 0.87 (3H, s, 30- CH_3), 0.90 (3H, d, J 6.1, 21- CH_3), 0.98 (3H, s, 19- CH_3), 1.00 (3H, s, 28- CH_3)], 1.81 – 3.00 [13H, m, containing 24- CH_2 , piperazine $\text{CH}_2 \times 2$, 2.94 (2H, br, s, CH_2 adjacent to NH)], 3.23 (1H, dd, J 11.3, 4.7, 3-CH); δ_{C} (75 MHz, CDCl_3) 15.4 (29- CH_3), 15.8 (18- CH_3), 18.3 (6- CH_2), 18.7 (21- CH_3), 19.1 (19- CH_3), 21.0 (11- CH_2), 23.3 (23- CH_2), 24.3 (30- CH_3), 26.5 (7- CH_2), 27.9 (2- CH_2), 28.0 (28- CH_3), 28.2 (16- CH_2), 30.8 (15- CH_2), 31.0 (12- CH_2), 34.0 (22- CH_2), 35.6 (1- CH_2), 36.3 (20-CH), 37.0 (10-quaternary C), 38.9 (4-quaternary C), 44.5 (13-quaternary C), 45.8 (piperazine $\text{CH}_2 \times 2$, adjacent to free NH), 50.3 (5-CH or 17-CH), 50.4 (5-CH or 17-CH), 53.6 (piperazine $\text{CH}_2 \times 2$, adjacent to tertiary N), 59.8 (24- CH_2), 79.0 (3-CH), 134.38 (8-quaternary C or 9-quaternary C), 134.44 (8-quaternary C or 9-quaternary C); m/z (ESI⁺) 471 [$\text{M} + \text{H}$]⁺ (4%), 42 (100); exact mass calculated for $\text{C}_{31}\text{H}_{55}\text{N}_2\text{O}$ [$\text{M} + \text{H}$]⁺ 471.4314, found 471.4309. Due to a slight discrepancy in integration values in the ¹H NMR spectrum, it is inferred that a trace amount of dihydrolanosterol **7** from the starting material is present in the product.

Assignments for piperazine derivatives are based on Zhang paper²⁹

3 β -Acetoxy-5 α -4,4,14-trimethyl-24-piperazino-*N*-2-hydroxyethyl-chol-8-ene **58**

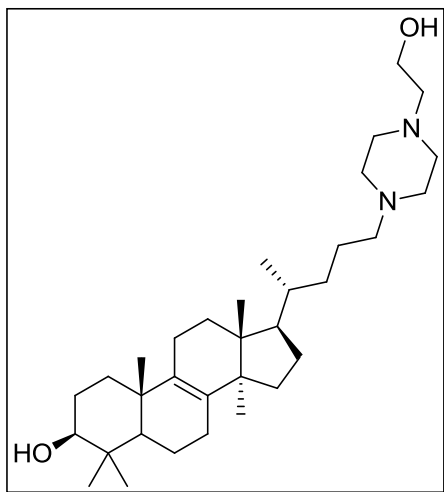


Sodium triacetoxyborohydride (0.161 g, 0.76 mM) was added to a solution of 3 β -acetoxy-5 α -4,4,14-trimethyl-chol-8-ene-24-al **21** (0.241 g, 0.54 mM) and 1-(2-hydroxyethyl)piperazine (0.070 g, 66 μL , 0.54 mM) in 1,2-dichloroethane (40 mL). Owing to the fact that 1-(2-hydroxyethyl)piperazine is a very viscous oil and could not be measured by microsyringe, the reagent was weighed in the following manner: a drop of 1-(2-hydroxyethyl)piperazine was placed in a vial and its

mass was recorded as 0.100 g. 10 mL of the reaction solvent was added and the amine was allowed to go into solution. 7 mL of this resulting solution was measured by syringe and transferred into the reaction vessel. The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate solution (20 mL) was added.

The reaction mixture was extracted into ethyl acetate (20 mL) and washed with brine (2×40 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield the desired product **58** as a light orange solid (0.286 g, 94%): mp $131 - 132$ °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3412, 2948, 1724; δ_{H} (300 MHz, CDCl_3) 0.69 (3H, s, 18-CH₃), 0.77 – 1.80 [35H, m, containing 21-CH₃, 28-CH₃, 29-CH₃, 30-CH₃], 1.00 (3H, s, 19-CH₃), 1.81 – 2.15 [8H, m, containing 2.05 (3H, s, OAc, CH₃)], 2.24 – 2.37 (2H, m, 24-CH₂), 2.38 – 2.64 (10H, m, containing piperazine N-CH₂ \times 4 and N-CH₂ of hydroxyethyl moiety), 3.28 (1H, br s, CH₂-OH), 3.62 (2H, t, J 5.4 CH₂-OH), 4.50 (3H, dd, J 11.2, 4.7, 3-CH); δ_{C} (75 MHz, CDCl_3) 15.7 (18-CH₃), 16.5 (29-CH₃), 18.1 (6-CH₂), 18.7 (21-CH₃), 19.1 (19-CH₃), 21.0 (11-CH₂), 21.3 (OAc, CH₃), 23.5 (23-CH₂), 24.16 (2-CH₂), 24.23 (30-CH₃), 26.4 (7-CH₂), 27.9 (28-CH₃), 28.2 (16-CH₂), 30.8 (15-CH₂), 30.9 (12-CH₂), 34.0 (22-CH₂), 35.3 (1-CH₂), 35.3 (quaternary C), 36.3 (20-CH), 36.8 (10-quaternary C), 43.5 (13-quaternary C), 49.5 (14-quaternary C), 50.3 (5-CH or 17-CH), 50.5 (5-CH or 17-CH), 52.8 (piperazine CH₂ \times 2), 53.2 (piperazine CH₂ \times 2), 57.7, 59.2, 59.3 (24-CH₂ or hydroxyethyl N-CH₂ or hydroxyethyl O-CH₂), 80.9 (3-CH), 133.3 (8-quaternary C or 9-quaternary C), 133.5 (8-quaternary C or 9-quaternary C), 170.0 (OAc, C=O, quaternary C); m/z (ESI⁺) 557 [$\text{M} + \text{H}$]⁺ (100%); exact mass calculated for $\text{C}_{35}\text{H}_{61}\text{N}_2\text{O}_3$ [$\text{M} + \text{H}$]⁺ 557.4682, found 557.4673.

3 β -Hydroxy-5 α -4,4,14-trimethyl-24-piperazino-*N*-2-hydroxyethyl-chole-8-ene **59**

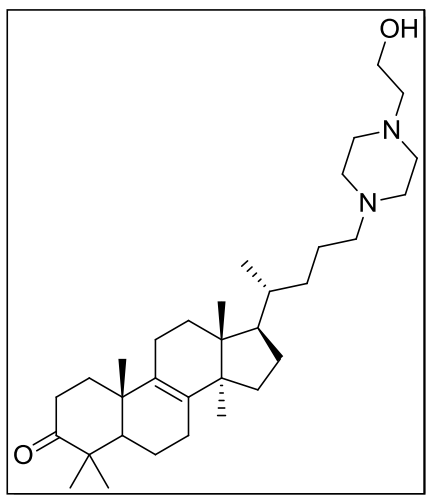


Sodium triacetoxyborohydride (0.248 g, 1.17 mM) was added to a solution of 3 β -hydroxy-5 α -4,4,14-trimethyl-chole-8-ene-24-al **25** (0.335 g, 0.84 mM) and 1-(2-hydroxyethyl)piperazine (0.10 mL, 0.84 mM) in 1,2-dichloroethane (50 mL). In this case, 0.1 mL of 1-(2-hydroxyethyl)piperazine was measured using a standard 1 mL plastic syringe. The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate solution (40 mL) was added. The reaction mixture was extracted into

ethyl acetate (20 mL) and washed with brine (2×50 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield the desired product **59** as an off white solid (0.380 g, 88%): mp $175 - 178$ °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3430,

2945; δ_{H} (300 MHz, CDCl_3) 0.69 (3H, s, 18- CH_3), 0.71 – 1.80 [44H, m, containing 0.81 (3H, s, 29- CH_3), 0.87 (3H, s, 30- CH_3), 0.90 (3H, d, J 6.1, 21- CH_3), 0.98 (3H, s, 19- CH_3), 1.00 (3H, s, 28- CH_3)], 1.81 – 2.13 (5H, m), 2.20 – 2.35 (2H, m, 24- CH_2), 2.36 – 2.62 (8H, m, containing piperazine $\text{N-CH}_2 \times 2$ and N-CH_2 of hydroxyethyl moiety), 3.23 (1H, dd, J 11.1, 4.6, 3-CH) 3.52 – 3.66 (2H, m, $\underline{\text{CH}_2\text{-OH}}$); δ_{C} (75 MHz, CDCl_3) 15.4 (29- CH_3), 15.7 (18- CH_3), 18.3 (6- CH_2), 18.8 (21- CH_3), 19.2 (19- CH_3), 21.0 (11- CH_2), 23.6 (23- CH_2), 24.3 (30- CH_3), 26.5 (7- CH_2), 27.8 (2- CH_2), 28.0 (28- CH_3), 28.2 (16- CH_2), 30.8 (15- CH_2), 31.0 (12- CH_2), 34.0 (22- CH_2), 35.6 (1- CH_2), 36.4 (20-CH), 36.4 (10-quaternary C), 38.9 (4-quaternary C), 44.5 (13-quaternary C), 49.8 (14-quaternary C), 50.3 (5-CH or 17-CH), 50.4 (5-CH or 17-CH), 52.8 (piperazine $\text{CH}_2 \times 2$), 53.3 (piperazine $\text{CH}_2 \times 2$), 57.7, 59.2, 59.3 (24- CH_2 or hydroxyethyl N-CH_2 or hydroxyethyl O-CH_2), 79.0 (3-CH), 134.3 (8-quaternary C or 9-quaternary C), 134.4 (8-quaternary C or 9-quaternary C); m/z (ESI^+) 515 $[\text{M} + \text{H}]^+$ (100%); exact mass calculated for $\text{C}_{33}\text{H}_{59}\text{N}_2\text{O}_2$ $[\text{M} + \text{H}]^+$ 515.4577, found 515.4589.

3-Keto-5 α -4,4,14-trimethyl-24-piperazino-*N*-2-hydroxyethyl-chole-8-ene **60**

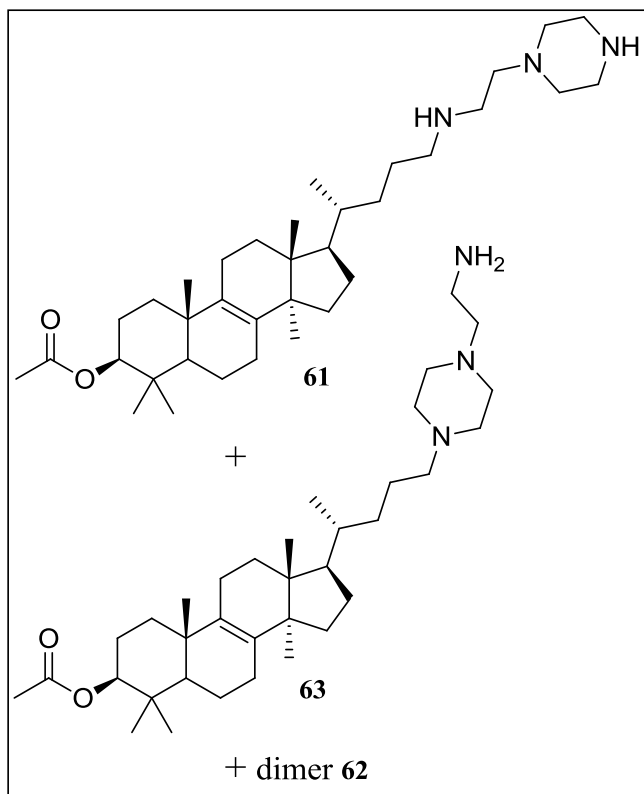


Sodium triacetoxyborohydride (0.074 g, 0.35 mM) was added to a solution of 3 β -keto-5 α -4,4,14-trimethyl-chole-8-ene-24-al **29** (0.100 g, 0.25 mM) and 1-(2-hydroxyethyl)piperazine (0.032 g, 0.31 μL , 0.25 mM) in 1,2-dichloroethane (30 mL). The reagent was weighed in the following manner: a drop of 1-(2-hydroxyethyl)piperazine was placed in a vial and its mass was recorded as 0.100 g. 10 mL of the reaction solvent was added and the amine was allowed to go into solution.

3.2 mL of this resulting solution was measured by syringe and transferred into the reaction vessel. The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate solution (15 mL) was added. The reaction mixture was extracted into ethyl acetate (20 mL) and washed with brine (2×20 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield the desired product **60** an orange solidifying oil (0.092 g, 72%): $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3394, 2946, 1707; δ_{H} (300 MHz, CDCl_3) 0.60 – 2.18 [44H,

m, containing 0.72 (3H, s, 18-CH₃), 0.89 (3H, s, 30-CH₃), 0.91 (3H, d, *J* 6.3, 21-CH₃), 1.07 (3H, s, 29-CH₃), 1.09 (3H, s, 28-CH₃), 1.12 (3H, s 19-CH₃), 2.25–2.95 (12H, m, containing 2.33 (2H, t, *J* 7.7, 24-CH₂), piperazine N-CH₂ × 4 and N-CH₂ of hydroxyethyl moiety), 3.63 (2H, t, *J* 5.4, CH₂-OH); δ_C (75 MHz, CDCl₃) 15.9 (29-CH₃), 18.70 (18-CH₃ or 21-CH₃), 18.74 (18-CH₃ or 21-CH₃), 19.4 (6-CH₂), 21.1 (11-CH₂), 21.3 (19-CH₃), 23.4 (23-CH₂), 24.3 (30-CH₃), 26.2 (28-CH₃), 26.3 (7-CH₂), 28.2 (22-CH₂), 29.7 (15-CH₂ or 12-CH₂), 30.9 (15-CH₂ or 12-CH₂), 33.9 (22-CH₂), 34.6 (1-CH₂), 36.1 (2-CH₂), 36.3 (20-CH), 36.9 (4-quaternary C), 44.5 (13-quaternary C), 47.4 (10-quaternary C), 49.9 (14-quaternary C), 50.3 (5-CH or 17-CH), 51.2 (5-CH or 17-CH), 52.6 (piperazine CH₂ × 2), 53.0 (piperazine CH₂ × 2), 57.6, 59.1, 59.2 (24-CH₂ or hydroxyethyl N-CH₂ or hydroxyethyl O-CH₂), 133.2 (8-quaternary C or 9-quaternary C), 135.3 (8-quaternary C or 9-quaternary C), 218.0 (3-quaternary C, C=O); *m/z* (ESI⁺) 513 [M + H]⁺ (100%); exact mass calculated for C₃₃H₅₇N₂O₂ [M + H]⁺ 513.4420, found 513.4422.

3β-Acetoxy-5α-4,4,14-trimethyl-24-(2-(piperazin-1-yl)ethyl)amino-cholesterol-8-ene 61, 3β-Acetoxy-5α-4,4,14-trimethyl-24-piperazino-*N*-2-aminoethyl-cholesterol-8-ene 63 and dimer 62

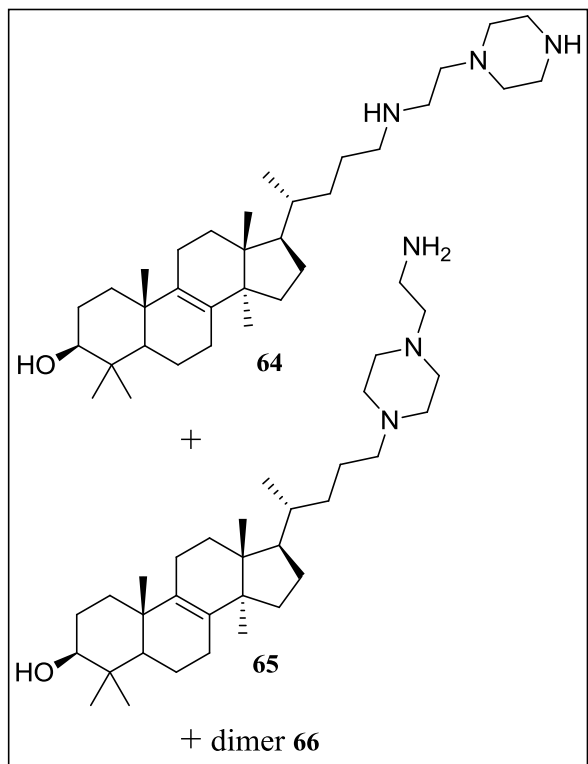


Sodium triacetoxyborohydride (0.131 g, 0.62 mM) was added to a solution of 3β-acetoxy-5α-4,4,14-trimethyl-cholesterol-8-ene-24-al **21** (0.196 g, 0.44 mM) and 1-(2-aminoethyl)piperazine (58 μL, 0.44 mM) in 1,2-dichloroethane (40 mL). The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate solution (20 mL) was added. The reaction mixture was extracted into ethyl acetate (20 mL) and washed with brine (2 × 40 mL). The organic extracts were then dried over magnesium sulfate and concentrated

under reduced pressure to yield a mixture of the desired product **61** regioisomer **63** and dimer

62 in an indecipherable ratio, as a light orange solid (0.232 g). All attempts to remove the minor regioisomer, by recrystallisation and chromatography, were unsuccessful: mp 125 – 128 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 2950, 1735, 1716; δ_{H} (300 MHz, CDCl_3) 0.68 (3H, s, 18- CH_3), 0.72 – 1.80 [42H, m, containing 21- CH_3 , 28- CH_3 , 29- CH_3 , 30- CH_3 and 1.00 (3H, s, 19- CH_3)], 1.81 – 2.13 [8H, m containing 2.05 (3H, s, OAc, CH_3)], 2.15 – 2.90 [12H, m, containing (2H, m, 24- CH_2), piperazine N- $\text{CH}_2 \times 4$ and N- CH_2 of aminoethyl moiety adjacent to tertiary N, for both regioisomers], 4.50 (1H, dd, J 11.2, 4.7, 3-CH); δ_{C} (75 MHz, CDCl_3) 15.7 (29- CH_3), 16.5 (29- CH_3), 18.1 (6- CH_2), 18.7 (21- CH_3), 19.2 (19- CH_3), 21.0 (11- CH_2), 21.3 (OAc, CH_3), 23.6 (23- CH_2), 24.16 (2- CH_3), 24.23 (30- CH_2), 26.3 (7- CH_2), 27.9 (28- CH_3), 28.2 (16- CH_2), 29.7 (CH_2), 30.8 (15- CH_2), 31.0 (12- CH_2), 34.0 (22- CH_2), 35.3 (1- CH_2), 36.3 (quaternary C), 36.4 (20-CH), 36.8 (10-quaternary C), 37.8 (quaternary C), 38.5 ($\text{H}_2\text{N-CH}_2$ of aminoethyl chain in regioisomer **63**), 44.4 (13-quaternary C), 45.8 and 45.9 ($2 \times \text{CH}_2$ adjacent to free NH of piperazine ring in regioisomer **61**), 49.7 (14-quaternary C), 50.3 (5-CH or 17-CH), 50.5 (5-CH or 17-CH), 53.2 (piperazine N- $\text{CH}_2 \times 2$), 59.3 (N- CH_2 of aminoethyl chain, and or 24- CH_2 in regioisomer **63**, and or N- CH_2 of aminoethyl chain in regioisomer **61**), 80.9 (3-CH), 133.3 (8-quaternary C or 9-quaternary C), 133.5 (8-quaternary C or 9-quaternary C), 170.0 (OAc, C=O, quaternary C); m/z (ESI^+) 556 [$\text{M} + \text{H}]^+$ (52%), 249 (100); exact mass calculated for $\text{C}_{35}\text{H}_{62}\text{N}_3\text{O}_2$ [$\text{M} + \text{H}]^+$ 556.4842, found 556.4841, for compounds **61** and **63**; exact mass calculated for $\text{C}_{64}\text{H}_{108}\text{N}_3\text{O}_4$ [$\text{M} + \text{H}]^+$ 982.8261, found 982.8348, for dimer **62**.

3 β -Hydroxy-5 α -4,4,14-trimethyl-24-(2-(piperazin-1-yl)ethyl)amino-chol-8-ene **64, 3 β -Hydroxy-5 α -4,4,14-trimethyl-24-piperazino-*N*-2-aminoethyl-chol-8-ene **65** and dimer **66****

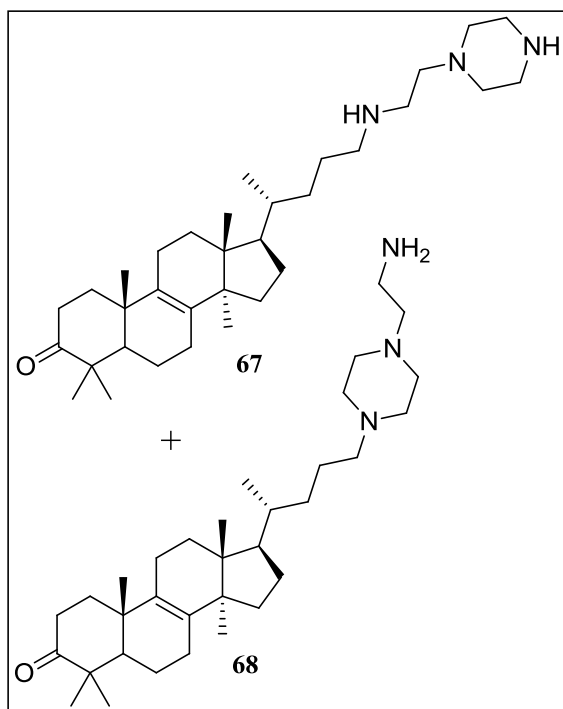


Sodium triacetoxyborohydride (0.236 g, 1.11 mM) was added to a solution of 3 β -hydroxy-5 α -4,4,14-trimethyl-chol-8-ene-24-al **25** (0.319 g, 0.79 mM) and 1-(2-aminoethyl)piperazine (0.1 mL, 0.79 mM) in 1,2-dichloroethane (50 mL). The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate solution (50 mL) was added. The reaction mixture was extracted into ethyl acetate (20 mL) and washed with brine (2 \times 40 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield a mixture of the desired product **64**,

regioisomer **65** and dimer **66**, in an indecipherable ratio as an off white solid (0.347 g). All attempts to remove the minor regioisomer, by recrystallisation and chromatography, were unsuccessful: mp 123 – 126 °C; ν_{max} (KBr)/cm⁻¹ 3413, 2945; δ_{H} (300 MHz, CDCl₃) 0.50 – 2.20 [46 H, m, containing 0.69 (3H, s, 18-CH₃), 0.81 (3H, s, 29-CH₃), 0.87 (3H, s, 30-CH₃), 0.90 (3H, d, *J* 6.0, 21-CH₃), 0.98 (3H, s, 19-CH₃), 1.00 (3H, s, 28-CH₃)], 2.21 – 2.90 [12 H, m, containing (2H, m, 24-CH₂), piperazine N-CH₂ \times 4 and N-CH₂ of aminoethyl moiety adjacent to tertiary N, for both regioisomers], 3.23 (1H, dd, *J* 11.3, 4.7, 3-CH); δ_{C} (75 MHz, CDCl₃) 15.4 (29-CH₃), 15.7 (18-CH₃), 18.3 (6-CH₂), 18.7 (21-CH₃), 19.1 (19-CH₃), 21.0 (11-CH₂), 24.3 (30-CH₃), 26.5 (7-CH₂), 27.9 (2-CH₂), 28.0 (28-CH₃), 28.2 (16-CH₂), 30.8 (15-CH₂), 31.0 (12-CH₂), 33.7 (22-CH₂), 35.6 (1-CH₂), 36.3 (20-CH), 36.4 (10-quaternary C), 38.9 (4-quaternary C), 43.4 (CH₂), 44.5 (13-quaternary C), 45.5 and 46.2 (2 \times CH₂ adjacent to free NH of piperazine ring in regioisomer), 49.8 (14-quaternary C), 50.3 (5-CH or 17-CH), 50.4 (5-CH or 17-CH), 53.3 (piperazine N-CH₂ \times 2), 59.3 (N-CH₂ of aminoethyl chain, and or 24-CH₂ in regioisomer **65**, and or N-CH₂ of aminoethyl chain in regioisomer **64**), 78.9 (3-CH), 134.3 (8-quaternary C or 9-quaternary C), 134.4 (8-quaternary C or 9-quaternary C); *m/z* (ESI⁺) 514 [M + H]⁺ (40%), 74 (100); exact mass calculated for

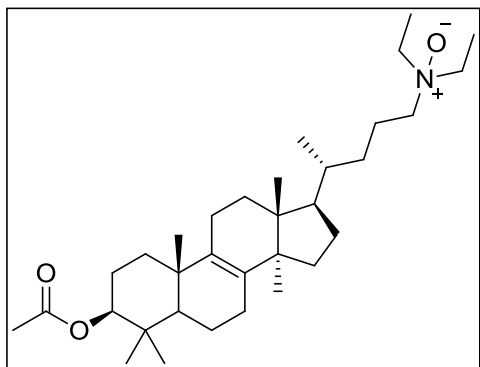
$C_{33}H_{60}N_3O$ $[M + H]^+$ 514.4736, found 514.4719, for compounds **64** and **65**; exact mass calculated for $C_{60}H_{104}N_3O_2$ $[M + H]^+$ 898.8050, found 898.8117, for dimer **66**.

3-Keto-5 α -4,4,14-trimethyl-24-(2-(piperazin-1-yl)ethyl)amino-chol-8-ene **67 and 3-Keto-5 α -4,4,14-trimethyl-24-piperazino-*N*-2-aminoethyl-chol-8-ene **68****



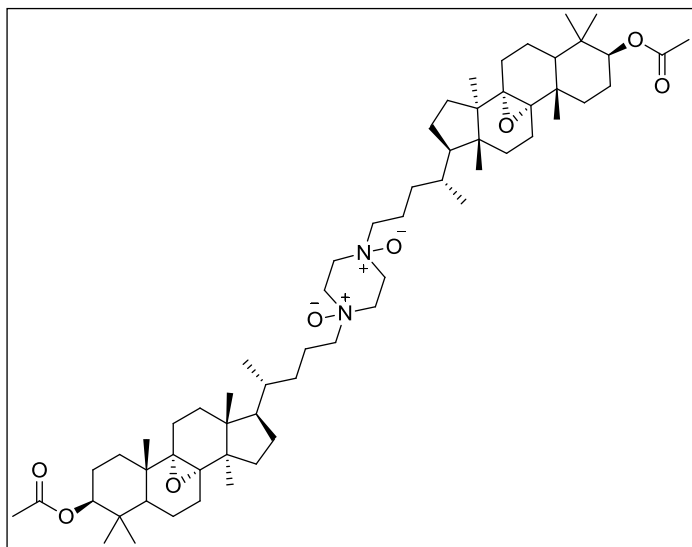
Sodium triacetoxyborohydride (0.089 g, 0.42 mM) was added to a solution of 3 β -keto-5 α -4,4,14-trimethyl-chol-8-ene-24-al **29** (0.120 g, 0.30 mM) and 1-(2-aminoethyl)piperazine (40 μ L, 0.30 mM) in 1,2-dichloroethane (50 mL). The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate solution (25 mL) was added. The reaction mixture was extracted into ethyl acetate (50 mL) and washed with brine (2 \times 50 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced

pressure to yield a mixture of the desired product **67** and regioisomer **68** in an indecipherable ratio, as an orange solidifying oil (0.098 g); δ_H (300 MHz, $CDCl_3$) 0.60 – 1.85 [34H, m, containing 0.72 (3H, s, 18- CH_3), 0.89 – 0.92 (9H, m, containing 21- CH_3 , 30- CH_3), 1.07 (3H, s, 29- CH_3), 1.09 (3H, s, 28- CH_3), 1.12 (3H, s 19- CH_3)], 1.86 – 2.17 (7H, m), 2.18 – 3.00 [16H, m, containing 24- CH_2 , piperazine N- $CH_2 \times 2$ and N- CH_2 of aminoethyl moiety]; δ_C (75 MHz, $CDCl_3$); 14.1 (CH_3), 15.9 (29- CH_3), 18.7 (18- CH_3 or 21- CH_3), 19.4 (6- CH_2), 21.1 (CH_3), 21.3 (19- CH_3), 24.3 (30- CH_3), 26.2 (28- CH_3), 26.3 (7- CH_2), 28.2 (22- CH_2), 29.7 (15- CH_2 or 12- CH_2), 30.9 (15- CH_2 or 12- CH_2), 34.6 (1- CH_2), 39.5 (H_2N-CH_2 of aminoethyl chain in regioisomer **68**), 36.1 (2- CH_2), 36.9 (4-quaternary C), 44.5 (13-quaternary C), 47.4 (10-quaternary C), 49.9 (14-quaternary C), 51.2 (5-CH or 17-CH), 59.2 (N- CH_2 of aminoethyl chain, and or 24- CH_2 in regioisomer **68**, and or N- CH_2 of aminoethyl chain in regioisomer **67**), 133.2 (8-quaternary C or 9-quaternary C), 135.3 (8-quaternary C or 9-quaternary C), 217.9 (3-quaternary C, C=O); m/z (ESI $^+$) 512 $[M + H]^+$ (38%), 56 (100); exact mass calculated for $C_{33}H_{58}N_3O$ $[M + H]^+$ 512.4580, found 512.4576, for compounds **67** and **68**.

N-Oxide Study**3 β -Acetoxy-5 α -4,4,14-trimethyl-24-diethylamino-chol-8-ene-N-oxide **69****

m-CPBA (0.062 g, 0.36 mM), was added to a stirring solution of 3 β -acetoxy-5 α -4,4,14-trimethyl-24-diethylamino-chol-8-ene **43** (0.128 g, 0.26 mM) in dichloromethane (20 mL). The solution was stirred at room temperature for 24 h.¹ The solution was twice filtered by gravity through a fluted filter paper to remove excess *m*-CPBA. The organic layer was then

washed with saturated aqueous sodium bicarbonate solution (3 \times 20 mL) and brine (1 \times 20 mL). The solution was dried over magnesium sulfate, and concentrated under reduced pressure to yield the crude product as an orange solidifying oil (0.166 g). The crude product was recrystallised from ethyl acetate to yield the desired compound **69** as a light orange solid (0.056 g, 34%): mp 185 – 188 °C; ν_{max} (KBr)/cm⁻¹ 2952, 1735; δ_{H} (300 MHz, CDCl₃) 0.69 (3H, s, 18-CH₃), 0.78 – 1.03 [15H, m, containing 21-CH₃, 28-CH₃, 29-CH₃, 30-CH₃, 1.00 (3H, s, 19-CH₃)], 1.05 – 1.80 (24H, m), 1.81 – 2.15 [8H, m, containing 2.05 (3H, s, OAc, CH₃)], 3.20 – 3.40 (2H, m, 24-CH₂), 3.42 – 3.57 (4H, m, diethylamine CH₂ \times 2), 4.50 (1H, dd, *J* 11.6, 4.5, 3-CH); δ_{C} (75 MHz, CDCl₃) 8.4 (diethylamine CH₃ \times 2), 15.8 (18-CH₃), 16.5 (29-CH₃), 18.1 (6-CH₂), 18.6 (21-CH₃), 19.2 (19-CH₃), 19.6 (CH₂), 21.0 (11-CH₂), 21.3 (23-CH₃), 24.1 (2-CH₂), 24.2 (30-CH₃), 27.9 (28-CH₃), 28.2 (16-CH₂), 30.7 (15-CH₂), 31.0 (12-CH₂), 35.3 (1-CH₂), 36.2 (20-CH), 36.9 (10-quaternary C), 37.8 (4-quaternary C), 44.6 (13-quaternary C), 49.8 (14-quaternary C), 50.4 (5-CH or 17-CH), 50.5 (5-CH or 17-CH), 59.2, 63.8, 70.4 (24-CH₂ or either of diethylamine CH₂ \times 2), 80.9 (3-CH), 134.3 (8-quaternary C or 9-quaternary C), 134.4 (8-quaternary C or 9-quaternary C), 171.0 (quaternary C, OAc, C=O); *m/z* (ESI⁺) 516 [M + H]⁺ (100%); exact mass calculated for C₃₃H₅₈NO₃ [M + H]⁺ 516.4417, found 516.4423.

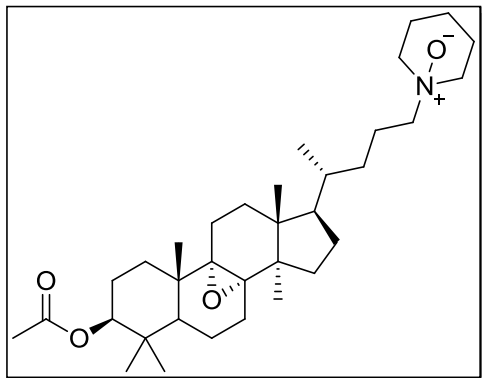
3 β -Acetoxy-5 α -4,4,14-trimethyl-24-piperazino-chol-8,9-epoxide-*N*-oxide dimer **71**

m-CPBA (0.067 g, 0.39 mM) was added to a solution of 3 β -acetoxy-5 α -4,4,14-trimethyl-24-piperazino-chol-8-ene dimer **56** (0.100 g, 0.19 mM) in dichloromethane (50 mL). The reaction mixture was stirred for at room temperature for 24 h.¹ The solution was twice filtered by gravity through a fluted filter paper to remove excess *m*-CPBA. The organic layer was then washed with

saturated aqueous sodium bicarbonate solution (4 \times 20 mL) and brine (1 \times 20 mL). The organic extract was dried over magnesium sulphate and concentrated under reduced pressure. The crude material was recrystallized from the minimum volume of hot ethyl acetate to yield product **71** as a yellow solid (0.060 g), among other minor products: mp 155 – 156 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3435, 2954, 1736; δ_{H} (300 MHz, CDCl_3) 0.60 – 2.20 [47H, m, containing 0.69 (3H, s, 18-CH₃), 21-CH₃, 28-CH₃, 29-CH₃, 30-CH₃, 1.00 (3H, s, 19-CH₃), 2.05 (3H, s, OAc, CH₃)], 2.82 – 3.55 (6H, m, 24-CH₂ and piperazine CH₂ \times 2 adjacent to *N*-oxide), 4.22 (2H, br s), 4.48 (1H, dd, *J* 24.2, 4.3, 3-CH); δ_{C} (75 MHz, CDCl_3) 15.8 (18-CH₃), 16.2 (29-CH₃), 18.1 (6-CH₂), 18.6 (21-CH₃), 19.2 (19-CH₃), 21.0 (11-CH₂), 21.3 (OAc, CH₃), 23.3 (23-CH₂), 24.15 (2-CH₂), 24.24 (30-CH₃), 26.4 (7-CH₂), 27.9 (28-CH₃), 28.5 (16-CH₂), 30.8 (15-CH₂), 30.9 (12-CH₂), 35.2 (1-CH₂), 36.8 (10-quaternary C), 37.8 (4-quaternary C), 44.5 (13-quaternary C), 49.8 (14-quaternary C), 50.2 (5-CH or 17-CH), 50.5 (5-CH or 17-CH), 59.1 (24-CH₂), 67.8 (8-quaternary C or 9-quaternary C), 70.4 (8-quaternary C or 9-quaternary C), 80.9 (3-CH), 171.0 (quaternary C, OAc, C=O);

Other peaks in the ^{13}C NMR spectrum indicate a mixture of minor products is present. The following peaks could not be accounted for in the structure of the major product: 16.24 (CH₃), 16.3 (CH₂), 16.5 (CH₃), 17.1 (CH₃), 18.8 (CH₃), 19.1 (CH₂), 19.9 (CH₃), 21.4 (CH₂), 23.5 (CH₂), 26.8 (CH₂), 28.3 (CH₃), 31.8 (CH₂), 32.5 (CH₂), 32.8 (CH₂), 36.2 (CH₃), 36.3 (CH₃), 41.8 (CH₃), 48.1 (CH₃), 71.6 (CH₂), 80.4 (CH), 134.3 (quaternary C).

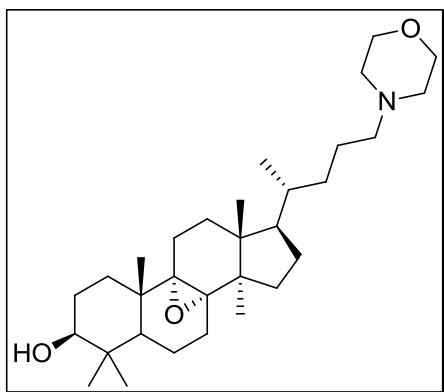
Exact mass calculated for $\text{C}_{62}\text{H}_{103}\text{N}_2\text{O}_8$ [$\text{M} + \text{H}$]⁺ 1003.7714, found 1003.7683.

3 β -Acetoxy-5 α -4,4,14-trimethyl-24-piperidino-chol-8,9-epoxide-*N*-oxide **70**

m-CPBA (0.244 g, 1.45 mM) was added to a solution of 3 β -acetoxy-5 α -4,4,14-trimethyl-24-piperidino-chol-8-ene **41** (0.362 g, 0.71 mM) in dichloromethane (100 mL). The reaction mixture was stirred for at room temperature for 24 h.¹ The solution was twice filtered by gravity through a fluted filter paper to remove excess *m*-CPBA. The organic layer was then washed with saturated

aqueous sodium bicarbonate solution (4 \times 50 mL) and brine (2 \times 50 mL). Several emulsions formed in the washing process. The organic extract was dried over magnesium sulphate and concentrated under reduced pressure to yield the crude product **70** (0.170 g) as an orange oil. Multiple recrystallisations from hexane and ethyl acetate could not remove the excess *m*-CPBA in this case. The analysis described below is taken on the sample with *m*-CPBA present, as indicated by distinctive aromatic signals in the 7.30 – 8.10 ppm region of the ¹H NMR spectrum.

δ_H (300 MHz, CDCl₃) 0.50 – 2.10 [52H, m, containing 18-CH₃, 19-CH₃, 21-CH₃, 28-CH₃, 29-CH₃, 30-CH₃, 2.05 (3H, s, OAc, CH₃)], 2.20 – 2.40 (1H, m), 3.15 (1H, br s), 3.38 – 3.62 (1H, m), 3.75 (1H, br s), 4.40 – 4.55 (1H, m, 3-CH); exact mass calculated for C₃₂H₅₈NO₄ [M + H]⁺ 544.4366, found 544.4367.

Test Reductive Amination**3 β -Hydroxy-5 α -4,4,14-trimethyl-24-morpholino-chol-8,9-epoxide **73****

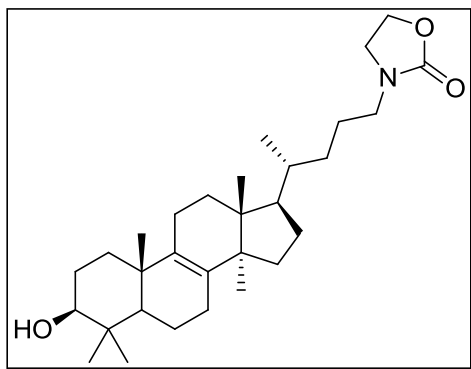
Sodium triacetoxyborohydride (0.071 g, 0.33 mM) was added to a solution of morpholine (21 μ L, 0.24 mM) and a sample containing ~ 30% 3 β -hydroxy-5 α -4,4,14-trimethyl-chol-8,9-epoxide-24-al **72** (0.100 g, 0.24 mM) in 1,2-dichloroethane (50 mL). The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate solution (30 mL). The reaction mixture was extracted into ethyl

acetate and washed with brine (2×40 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield a yellow oil (0.061 g), consisting of numerous unidentifiable products. The crude material was not further purified. Characteristic signals for the desired product **73** are described below:

$\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3400, 2929; δ_{H} (300 MHz, CDCl_3) 0.60 – 4.00 [53H, m, containing 0.78 (3H, s, 18- CH_3), 19- CH_3 , 21- CH_3 , 26- CH_3 , 27- CH_3 , 28- CH_3 , 29- CH_3 , 30- CH_3 , 24- CH_2 , 3.21 (1H, dd, J 11.2, 4.2, 3-CH), 3.82 (4H, br s, morpholine $\text{CH}_2 \times 2$)]; δ_{C} (75 MHz, CDCl_3) 15.1 (29- CH_3), 16.3 (18- CH_3), 16.5 (6- CH_2), 17.0 (19- CH_3), 19.9 (30- CH_3), 21.5 (11- CH_2), 23.5 (2- CH_2), 26.9 (12- CH_2), 27.1 (CH_2), 28.25 (28- CH_3), 28.32 (CH_2), 28.4 (16- CH_2), 29.7 (CH_2), 31.9 (15- CH_2), 32.9 (1- CH_2), 36.0 (20-CH), 37.8 (4-quaternary C), 38.5 (10-quaternary C), 41.8 (5-CH), 43.6 (13-quaternary C), 48.1 (17-CH), 48.9 (14-quaternary C), 52.6 (morpholine N- $\text{CH}_2 \times 2$), 65.6 (morpholine O- $\text{CH}_2 \times 2$), 68.1 (8-quaternary C), 70.7 (9-quaternary C), 78.5 (3-CH); m/z (ESI^+) 488 [$\text{M} + \text{H}]^+$ (100%); exact mass calculated for $\text{C}_{31}\text{H}_{54}\text{NO}_3$ [$\text{M} + \text{H}]^+$ 488.4104, found 488.4103.

Attempted Reductive Aminations

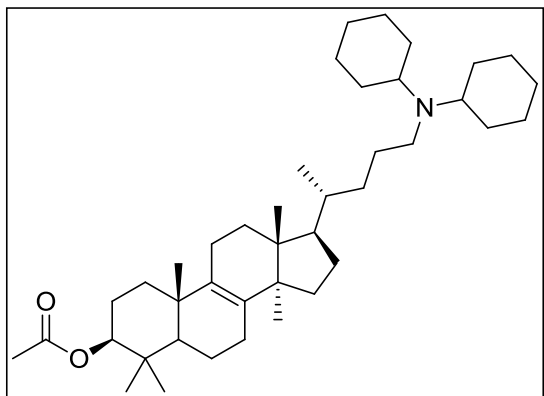
3 β -Hydroxy-5 α -4,4,14-trimethyl-24-oxazolodinone-chol-8-ene **53**



Sodium triacetoxyborohydride (0.126 g, 0.59 mM) was added to a solution of 3 β -hydroxy-5 α -4,4,14-trimethyl-chol-8-ene-24-al **25** (0.170 g, 0.42 mM) and oxazolodinone (0.037 g, 0.42 mM) in 1,2-dichloroethane (30 mL). The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate solution (15 mL). The reaction mixture was extracted into ethyl acetate and washed with brine (2×20 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield the crude product as a white solid (0.168 g). By inspection of the ^1H NMR spectrum, the crude product consisted of 75% starting aldehyde **25** with trace (3%) oxazolodinone signals at 3.64 and 4.45 ppm, and no unambiguous evidence of product formation.

The reaction was repeated, using the crude material from the first attempt, however, with the reaction time increased to 24 h. In this case, the product consisted of 55% starting aldehyde **25**, with no increase in oxazolodinone signals.

3 β -Acetoxy-5 α -4,4,14-trimethyl-24-dicyclohexylamino-chol-8-ene **51**



Sodium triacetoxyborohydride (0.100 g, 0.47 mM) was added to a solution of 3 β -acetoxy-5 α -4,4,14-trimethyl-chol-8-ene-24-al **21** (0.150 g, 0.34 mM) and dicyclohexylamine (68 μ L, 0.34 mM) in 1,2-dichloroethane (20 mL). The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate solution (20 mL)

was added. The reaction mixture was extracted into ethyl acetate (30 mL) and washed with brine (2 \times 20 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield a mixture of the desired product **51** and both aldehyde **21** (25%) and amine starting materials.

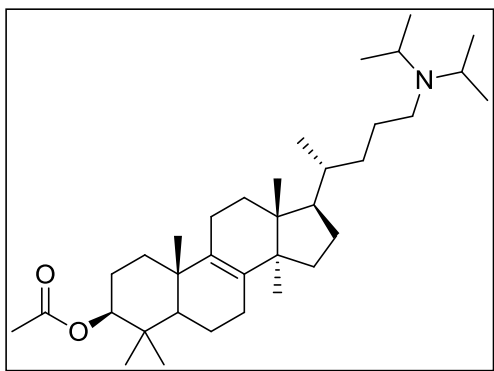
The reaction was repeated using 1.5 equivalents of dicyclohexylamine and a reaction time of 3 h in an attempt to consume all of the aldehyde starting material. In this case, an increase in aldehyde consumption was observed, with 14% aldehyde remaining, as indicated by the signal at 9.77 ppm in the ^1H NMR spectrum of the product. However, the product contained significant amounts of dicyclohexylamine, as indicated in the ^{13}C NMR spectrum.

δ_{H} (300 MHz, CDCl_3) 0.69 (3H, s, 18- CH_3), 0.80 – 2.15 [60H, m, containing 18- CH_3 , 19- CH_3 , 21- CH_3 , 28- CH_3 , 29- CH_3 , 30- CH_3 , 2.04 (3H, s, OAc, CH_3)], 2.30 – 2.61 (8H, m), 4.50 (1H, dd, J 11.6, 4.5, 3-CH); δ_{C} (75 MHz, CDCl_3) 15.7 (18- CH_3), 16.5 (29- CH_3), 18.1 (6- CH_2), 18.8 (21- CH_3), 19.2 (19- CH_3), 21.0 (11- CH_2), 21.3 (OAc, CH_3), 24.16 (2- CH_2), 24.22 (30- CH_3), 26.4 (7- CH_2), 26.5 (CH_2), 27.9 (28- CH_3), 28.1 (16- CH_2), 28.4 (CH_2), 30.8 (15- CH_2), 31.0 (12- CH_2), 31.6 (CH_2), 31.7 (CH_2), 33.8 (22- CH_2), 35.3 (1- CH_2), 36.3 (20-CH), 36.8 (10-quaternary C), 37.8 (4-quaternary C), 44.4 (13-quaternary C), 47.0 (CH_2), 49.8 (14-quaternary C), 50.5 (5-CH or 17-CH), 58.0 (CH), 80.9 (3-CH), 134.2 (8-quaternary C or 9-quaternary C), 134.5 (8-quaternary C or 9-quaternary C), 170.9 (quaternary C, OAc, $\text{C}=\text{O}$);

m/z (ESI⁺) 608 [M + H]⁺ (21 %), 182 (100); exact mass calculated for C₄₁H₇₀NO₂ [M + H]⁺ 608.5407, found 608.5410.

The following peaks in the ¹³C NMR spectrum are attributable to excess dicyclohexylamine: 25.3, 26.2, 34.3, 53.0.

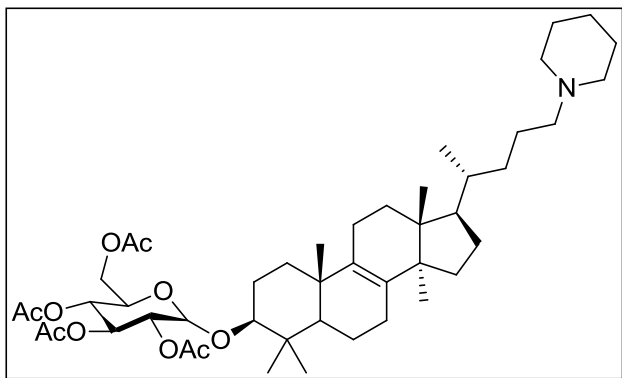
3β-Acetoxy-5α-4,4,14-trimethyl-24-diisopropylamino-chol-8-ene 52



Sodium triacetoxyborohydride (0.67 g, 0.32 mM) was added to a solution of 3β-acetoxy-5α-4,4,14-trimethyl-chol-8-ene-24-al **21** (0.100 g, 0.23 mM) and diisopropylamine (32 μL, 0.23 mM) in 1,2-dichloroethane (20 mL). The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate solution (20 mL) was added. The reaction mixture was extracted into ethyl acetate (30 mL) and washed with brine (2 × 20 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield the crude product as a black solidifying oil (0.104 g) consisting of 55% starting aldehyde **21**, with no evidence of product formation.

Attempted Glycosylations

Attempted Synthesis of 3β-tetra-*O*-acetyl-β-D-glucopyranose-5α-4,4,14-trimethyl-24-piperadino-chol-8-ene 74



Triethylamine (0.004 g, 0.04 mM) in dichloromethane (20 mL) was added to a mixture of penta-*O*-acetyl-β-D-glucopyranose (0.027 g, 0.07 mM) and 3β-hydroxy-5α-4,4,14-trimethyl-24-piperadino-chol-8-ene **42** (0.050 g, 0.11 mM) under a nitrogen atmosphere. Boron trifluoride diethyl etherate (0.025 g, 22 μL, 0.18 mM) in dichloromethane (5 mL) was then added to the reaction solution. The additions took place over a 30 minute period.^{30,31} The reaction mixture was stirred at room

temperature for 24 h. Saturated aqueous sodium bicarbonate (20 mL) was added to the reaction mixture, and the reaction mixture was then extracted with ethyl acetate (2×30 mL). The organic layer was dried over magnesium sulfate and concentrated under reduced pressure to yield the crude product as a dark orange solid (0.080 g). Analysis of the ^1H NMR spectrum of the crude material, in conjunction with the ^1H NMR spectra of the starting alcohol and sugar, showed that a mixture of the two starting materials was present, with no evidence of the desired glycosylation product **74** having been formed. Characteristic signals for the mixture of starting materials are described below:

$\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3372 (OH of **42**).

δ_{H} (300 MHz, CDCl_3) 2.02, 2.038, 2.039, 2.09, 2.12 ($5 \times \text{OAc}$, CH_3 of penta-*O*-acetyl- β -D-glucopyranose), 3.23 (1H, dd, J 11.5, 4.54, 3-CH of **42**), 5.72 (1H, d, J 8.3, 1-CH of penta-*O*-acetyl- β -D-glucopyranose).

δ_{C} (75 MHz, CDCl_3) 78.9 (3-CH of **42**), 168.9, 169.2, 169.4, 170.1, 170.6 ($5 \times$ quaternary C, C=O, of penta-*O*-acetyl- β -D-glucopyranose)

Method B:

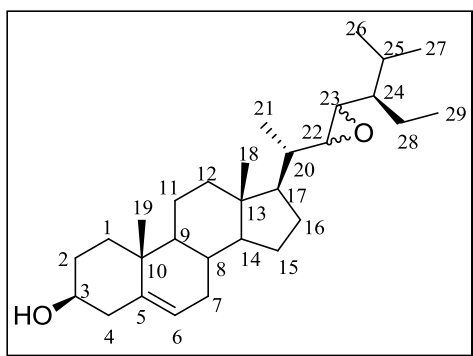
1-Bromo-1-deoxy-2,3,4,6-tetra-*O*-acetyl- β -D-glucose (0.066 g, 0.16 mM) was dissolved in acetonitrile (15 mL) with 3β -hydroxy-5 α -4,4,14-trimethyl-24-piperadino-chol-8-ene **42** (0.050, 0.11 mM) and 4 Å molecular sieves. The solution was cooled to 0 °C using an ice bath. Silver oxide (0.037 g, 0.16 mM) was added to the stirring solution in the dark. The reaction solution was stirred overnight, then filtered through a bed of Celite[®].³¹ The bed of Celite[®] was washed with acetonitrile (3×30 mL), and the solvent was removed under reduced pressure. Analysis of the ^1H NMR spectrum of the crude material, in conjunction with the ^1H NMR spectra of the starting alcohol and sugar, showed that a mixture of the two starting materials was present, with no evidence of the desired glycosylation product **74** having been formed. Characteristic signals for the mixture of starting materials are described below:

δ_{H} (300 MHz, CDCl_3) 2.04, 2.06, 2.10, 2.11 ($4 \times \text{OAc}$, CH_3 of 1-bromo-1-deoxy-2,3,4,6-tetra-*O*-acetyl- β -D-glucose), 3.24 (1H, dd, J 11.5, 4.4 of **42**), 6.62 (1H, d, J 4.0, 1-CH of 1-bromo-1-deoxy-2,3,4,6-tetra-*O*-acetyl- β -D-glucose)

3.4 Stigmasterol Chemistry

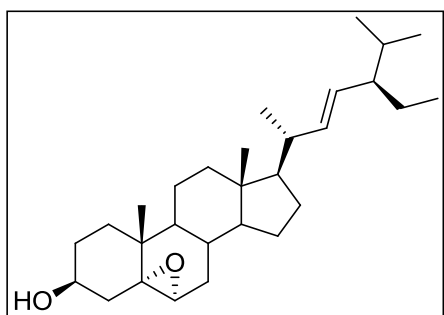
Attempted Selective Epoxidation

Stigmasterol 22-23 epoxide³² **76a** and **76b**



Stigmasterol **9** (0.050 g, 0.12 mM) was dissolved in dichloromethane (50 mL). *m*-CPBA (0.028 g, 0.16 mM) and sodium bicarbonate (0.021 g, 0.25 mM) powders were mixed together. Half of this mixture was added to the stirring stigmasterol solution at room temperature, and the second half was added at 0 °C over two 10 min periods respectively. The reaction mixture was then stirred for 3 h. The reaction mixture was filtered, washed with 10% sodium bicarbonate (4 × 20 mL) followed by brine (1 × 20 mL) and dried over magnesium sulfate to yield the crude product as a white solid (0.041 g). The crude material consisted of 30% starting material **9**, 60% product **77a** and 10% product **77b**, with no evidence of the formation of desired product **76a** and **76b**. Purification by column chromatography using hexane : ethyl acetate (90:10) led to the isolation of starting material stigmasterol **9** (0.010 g,) as the first fraction from the column. The second fraction from the column was a mixture of epoxides **77a** and **77b** in a 4:1 ratio (0.026 g, 51%).

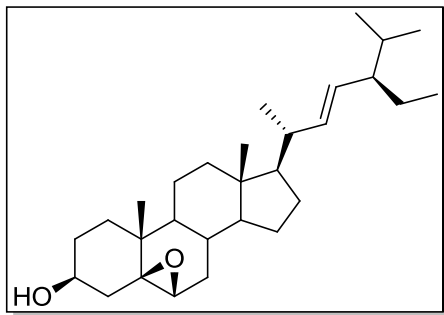
Stigmasterol 5,6 α -epoxide³³ **77a**



one of 22-CH or 23-CH).

δ_H (300 MHz, $CDCl_3$) 0.63 (3H, s, 18- CH_3), 0.71 – 0.89 [9H, m, containing 26- CH_3 , 27- CH_3 and 29- CH_3], 0.90 – 1.75 [37H, m, containing 1.00 (3H, d, *J* 6.6, 21- CH_3), 1.06 (19- CH_3)], 1.77 – 2.18 (5H, m), 2.90 (1H, d, *J* 4.4, 6-CH), 3.83 – 3.99 (1H, m, 3-CH), 5.00 (1H, dd, *J* 15.2, 8.6, one of 22-CH or 23-CH), 5.14 (1H, dd, *J* 15.2, 8.6,

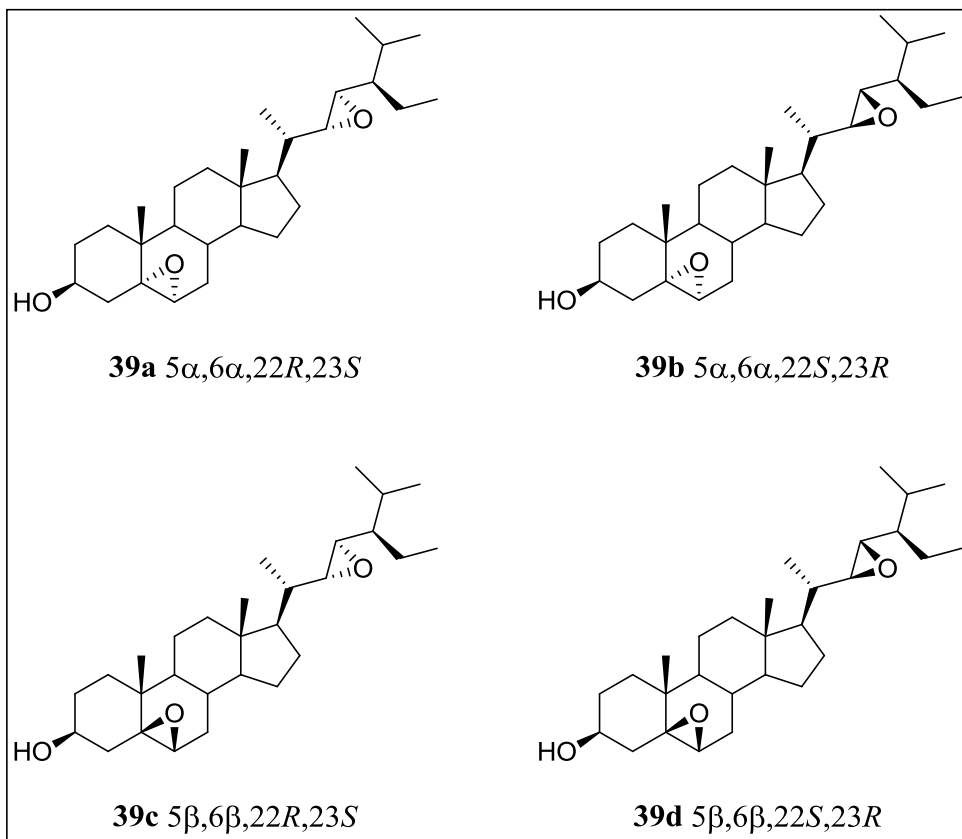
Stigmasterol 5,6 β -epoxide³³ 77b



δ_H (300 MHz, $CDCl_3$) 0.71 – 0.89 [9H, m, containing 0.78 (3H, s, 18- CH_3), 26- CH_3 , 27- CH_3 and 29- CH_3], 0.90 – 1.75 [37H, m, containing 1.00 (3H, d, J 6.6, 21- CH_3)], 1.77 – 2.18 (5H, m), 3.05 (1H, d, J 2.2, 6-CH), 3.67 – 3.78 (1H, m, 3-CH), 5.00 (1H, dd, J 15.2, 8.6, one of 22-CH or 23-CH), 5.14 (1H, dd, J 15.2, 8.6, one

of 22-CH or 23-CH).

5,6,22,23-Diepoxy stigmasterane³³ 39



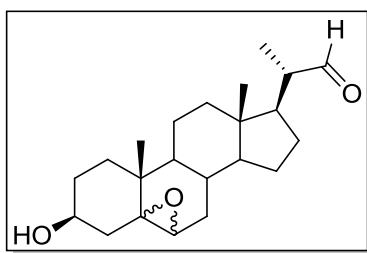
Stigmasterol **9** (0.100 g, 0.24 mM) was dissolved in dichloromethane (100 mL) and *m*-CPBA (0.155 g, 0.90 mM) was added at 0 °C. The reaction solution was allowed to warm to room temperature and stirred for 48 h. The reaction mixture was filtered and washed with 10% sodium bicarbonate (4 \times 50 mL) and brine (1 \times 50 mL). The organic solution was dried over magnesium sulfate and concentrated under reduced pressure to yield the crude product as a

white solid (0.136 g) which contained residual *m*-CPBA but was not further purified. Analysis of the ^1H NMR spectrum of the product shows the following ratio of the four diastereomers: **39a**: **39b**: **39c**: **39d** 3.7: 3.7: 1: 1.

Isomers **39a** and **39b**: δ_{H} (300 MHz, CDCl_3) 0.55 – 2.18 [42H, m, containing 0.61 (3H, m, 18- CH_3), 26- CH_3 , 27- CH_3 , 29- CH_3 , 21- CH_3 , 1.05 (3H, s, 19- CH_3)], 2.45 – 2.55 (3H, m, 22(*R*)-CH, 22(*S*)-CH, one of 23-CH), 2.72 – 2.77 (1H, m, one of 23-CH), 2.92 (1H, d, *J* 4.3, 6-CH), 3.87 – 3.98 (1H, m, 3-CH).

Isomers **39c** and **39d**, distinguishable signals: δ_{H} (300 MHz, CDCl_3) 2.45 – 2.55 (3H, m, 22(*R*)-CH, 22(*S*)-CH, one of 23-CH), 2.72 – 2.77 (1H, m, one of 23-CH), 3.08 (1H, d, *J* 1.9, 6-CH), 3.66 – 3.77 (1H, m, 3-CH).

Attempted synthesis of 3 β -hydroxy-5,6-epoxy-20(*S*)-methyl-pregnane-21-al **78**

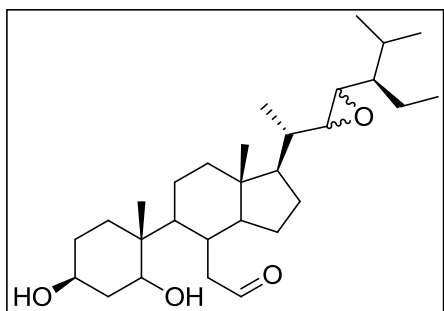


Diepoxide **39**, containing residual *m*-CPBA from the previous reaction, (0.136 g, 0.31 mM) was added to ground periodic acid (0.100 g, 0.44 mM) in dry ether (40 mL). The solution was stirred at 25 °C for 10 min, after which time water (20 mL) was added and the ether layer was separated. The ether layer was washed with water (2 × 20 mL) and brine (1 × 20 mL), dried over magnesium sulfate and concentrated under reduced pressure¹² to yield the crude product (0.132 g). Analysis of the ^1H NMR spectrum of the crude material showed no formation of the desired product **78**, given that the C-22,23 epoxide signals at 2.45 – 2.55 and 2.72 – 2.77 ppm were still present. The C-5,6 epoxide signals at 2.92 and 3.08 ppm diminished by ~ 80%, while there was evidence of an aldehyde formation due to a new broad singlet at 9.62 ppm, accounting for ~ 5%. There is also evidence of unidentified products in ^1H NMR spectrum, due to new signals at 3.55 and 4.13 ppm.

The reaction was repeated using the crude material from the previous attempt. In this instance, 2.86 equivalents of periodic acid were used and the reaction time was increased to 2 h. All other procedures remained identical and 0.127 g crude product was retrieved. Analysis of the ^1H NMR spectrum of the crude material showed no formation of the desired product

78, given that the C-22,23 epoxide signals at 2.45 – 2.55 and 2.72 – 2.77 ppm were still present. The C-5,6 epoxide had been consumed in the reaction and two new aldehyde signals at 9.62 and 9.71 ppm were observed. The crude mixture was purified by column chromatography using hexane : ethyl acetate 90:10 eluent, which led to the isolation of two products.

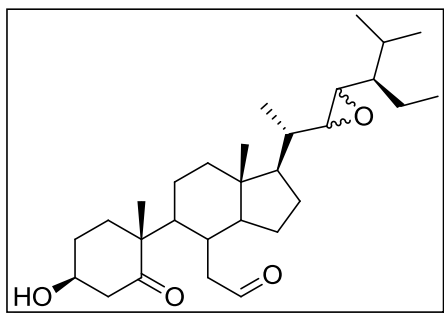
Stigmasterol 22,23-epoxy-5-ol-6-al **79a** and **79b**



The first product isolated from the column is being tentatively assigned as **79**. Characteristic signals for the assigned structure in the ^1H and ^{13}C NMR spectra are listed below:

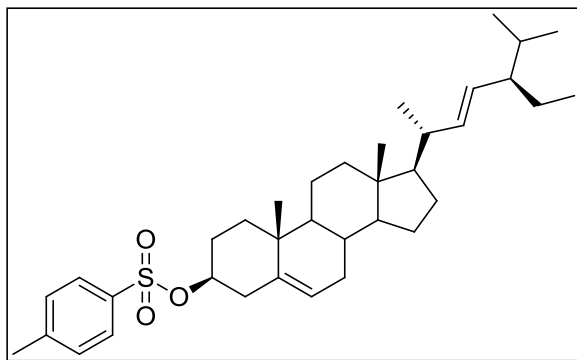
δ_{H} (300 MHz, CDCl_3) 2.45 – 2.55 (3H, m, 22-CHR, 22-CHS, one of 23-CH), 2.72 – 2.77 (1H, m, one of 23-CH), 3.49 (1H, d, J 9.5, 5-OH), 4.08 – 4.20 (1H, m, 3-CH), 9.71 (1H, t, J 2.8, 6-CH). δ_{C} (300 MHz, CDCl_3) 204.5 (aldehyde CH)

Stigmasterol 22,23-epoxy-5-one-6-al **80a** and **80b**



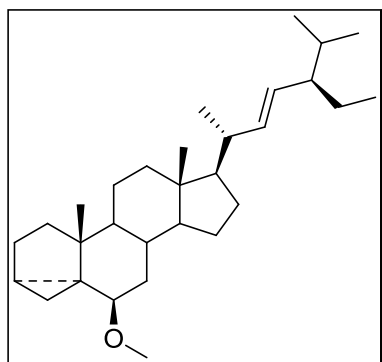
The second product isolated from the column is being tentatively assigned as **80**. Characteristic signals for the assigned structure in the ^1H and ^{13}C NMR spectra are listed below:

δ_{H} (300 MHz, CDCl_3) 2.45 – 2.55 (3H, m, 22-CHR, 22-CHS, one of 23-CH), 2.72 – 2.77 (1H, m, one of 23-CH), 3.11 (1H, apparent dt, J 13.7, 3.8, 2-CH₂), 9.62 (1H, br s, 6-CH); δ_{C} (300 MHz, CDCl_3) 202.7 (aldehyde CH), 217.3 (5-quaternary C, C=O).

Stigmasteryl Tosylate³³ 81

A solution of stigmasterol **9** (1.850 g, 4.48 mM), 4-DMAP (0.054 g, 0.45 mM), and *p*-toluenesulfonyl chloride (1.614 g, 8.46 mM) in pyridine (18.5 mL) was stirred at room temperature for 22 h. The reaction mixture was poured into 10% aqueous sodium bicarbonate (74 mL), and the precipitate was

filtered by suction. The resulting solid was washed with water (40 mL) and recrystallised from acetone to give the desired tosylate **81** as a white solid (1.930 g, 76%), mp 144 – 145 °C (literature,³³ 142 – 144 °C); $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 2957, 1664, 1191, 943; δ_{H} (300 MHz, CDCl_3) 0.68 (3H, s, 18- CH_3), 0.77 – 1.35 [24H, m, containing 26- CH_3 , 27- CH_3 and 29- CH_3 , 1.00 (3H, s, 19- CH_3), 1.02 (3H, d, J 6.6, 21- CH_3), 1.36 – 1.60 (9H, m), 1.62 – 1.87 (4H, m), 1.88 – 2.10 (3H, m), 2.21 – 2.32 (1H, m), 2.35 – 2.50 [4H, m, containing 2.45 (3H, s, Ar, CH_3)], 4.24 – 4.38 (1H, m, 3- $\text{CH}\alpha$), 5.01 (1H, dd, J 15.2, 8.6, one of 22-CH or 23-CH), 5.15 (1H, dd, J 15.2, 8.5, one of 22-CH or 23-CH), 5.30 (1H, d, J 4.8, 6-CH), 7.33 (2H, d, J 8.0, ArH), 7.80 (2H, d, J 8.3, ArH); δ_{C} (75 MHz, CDCl_3) 12.0 (29- CH_3), 12.2 (18- CH_3), 19.0 (19- CH_3), 19.1 (27- CH_3), 21.0 (11- CH_2), 21.1 (26- CH_3 or Ts, CH_3), 21.2 (26- CH_3 or Ts, CH_3), 21.6 (21- CH_3), 24.3 (15- CH_2), 25.4 (28- CH_2), 28.7 (CH_2), 28.9 (CH_2), 31.76 (8-CH), 31.84 (7- CH_2), 31.9 (CH), 36.4 (10-quaternary C), 36.9 (CH_2), 38.9 (CH_2), 39.6 (12- CH_2), 40.5 (20-CH), 42.2 (4-quaternary C), 50.0 (9-CH), 51.2 (CH), 55.9 (17-CH), 56.8 (14-CH), 82.4 (3-CH), 123.5 (6-CH), 127.6 (Ar-CH \times 2), 129.3 (23-CH), 129.7 (Ar-CH \times 2), 134.8 (Ar-quaternary C), 138.2 (22-CH), 138.9 (Ts, quaternary C, adjacent to S), 144.4 (5-quaternary C).

***i*-Stigmasterol methyl ether³³ 82**

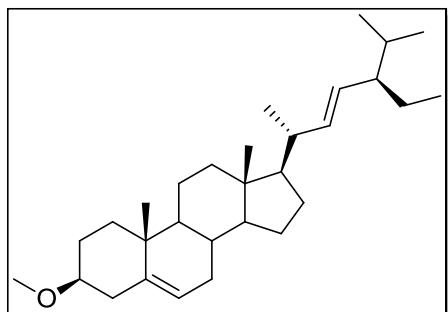
Tosylate **81** (1.542 g, 2.27 mM), and pyridine (0.70 mL, 8.22 mM) were dissolved in anhydrous methanol (37.5 mL) and heated under reflux for 6 h. The solution was evaporated, extracted into ethyl acetate (35 mL) and washed with water (2 \times 50 mL). The organic layer was washed with brine (1 \times 50 mL), dried over magnesium sulfate and concentrated

under reduced pressure to give the crude product as a yellow oily solid, as a mixture (4.15:1) of *i*-stigmasterol methyl ether **82** and stigmasterol methyl ether **83** (0.902 g). Purification by column chromatography using 98:2 hexane : ethyl acetate yielded the desired compound **82** as a yellow oil (0.572 g, 59%) as the first fraction from the column. Spectral details described below.

$\nu_{\max}(\text{film})/\text{cm}^{-1}$ 2955, 1456; δ_{H} (300 MHz, CDCl_3) 0.43 (1H, dd, J 8.0, 5.1, 3-CH), 0.61 – 0.67 (1H, m), 0.74 (3H, s, 18- CH_3), 0.75 – 0.94 (12H, m, containing 26- CH_3 , 27- CH_3 and 29- CH_3), 0.96 – 1.33 (13H, m, containing 19- CH_3 and 21- CH_3), 1.34 – 1.63 (8H, m), 1.64 – 1.83 (3H, m), 1.84 – 2.12 (3H, m), 2.76 (1H, t, J 2.6, 6-CH), 3.32 (3H, s, OMe, CH_3), 5.01 (1H, dd, J 15.1, 8.7, one of 22-CH or 23-CH), 5.15 (1H, dd, J 15.1, 8.6, one of 22-CH or 23-CH); δ_{C} (75 MHz, CDCl_3) 12.3 (CH_3), 12.4 (CH_3), 13.1 (CH_2), 19.0 (CH_3), 19.3 (CH_3), 21.1 (CH_3), 21.2 (CH_3), 21.5 (CH), 22.8 (CH_2), 24.3 (15- CH_2), 25.0 (CH_2), 25.4 (28- CH_2), 29.0 (CH_2), 30.5 (CH), 31.9 (CH), 33.4 (CH_2), 35.2 (CH_2), 35.3 (10-quaternary C), 40.3 (CH_2), 40.6 (20-CH), 42.7 (quaternary C), 43.4 (quaternary C), 48.1 (CH), 51.3 (CH), 56.2 (17-CH), 56.55 (OMe, CH_3), 56.61 (14-CH), 82.4 (CH), 129.2 (23-CH), 138.4 (22-CH).

The second fraction from the column was side product stigmasterol methyl ether **83**, retrieved in 20% yield as a clear oil (0.189 g). Spectral details described below.

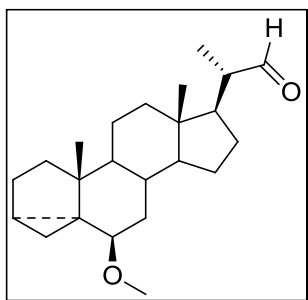
Stigmasterol methyl ether³³ **83**



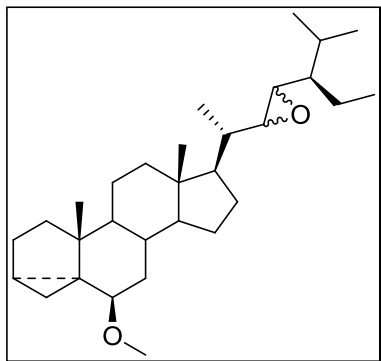
$\nu_{\max}(\text{film})/\text{cm}^{-1}$ 2938, 1460, 1105; δ_{H} (300 MHz, CDCl_3) 0.70 (3H, s, 18- CH_3), 0.74 – 1.34 [24H, m, containing 1.00 (3H, s, 19- CH_3), 1.02 (3H, d, J 6.6, 21- CH_3), 26- CH_3 , 27- CH_3 , and 29- CH_3], 1.35 – 1.62 (10H, m), 1.63 – 1.80 (1H, m), 1.81 – 2.24 (6H, m), 2.39 (1H, ddd, J 13.2, 4.7, 2.2), 2.98 – 3.13 (1H, m, 3-CH α), 3.35 (3H, s, OMe, CH_3), 5.02 (1H, dd J 15.2, 8.5, one of 22-CH or 23-CH), 5.16 (1H, dd, J 15.2, 8.4, one of 22-CH or 23-CH), 5.35 (1H, d, J 5.3 6-CH). δ_{C} (75 MHz, CDCl_3) 12.1 (29- CH_3), 12.3 (18- CH_3), 19.0 (19- CH_3), 19.4 (27- CH_3), 21.06 (11- CH_2), 21.10 (26- CH_3 or 21- CH_3), 21.2 (26-

CH₃ or 21-CH₃), 24.4 (15-CH₂), 25.4 (28-CH₂), 28.0 (CH₂), 28.9 (CH₂), 31.88 (8-CH), 31.94 (7-CH₂), 36.9 (10-quaternary C), 37.2 (CH₂), 38.7 (CH₂), 39.7 (12-CH₂), 40.5 (20-CH), 42.2 (4-quaternary C), 50.2 (9-CH), 51.2 (CH), 55.6 (OMe, CH₃), 55.9 (17-CH), 56.9 (14-CH), 80.4 (3-CH), 121.6 (6-CH), 129.3 (23-CH), 138.3 (22-CH), 140.9 (5-quaternary C).

20(*S*)-Methyl-6(*R*)-methoxy-3,5-cyclo-pregnane-21-al ^{36,37} **85**

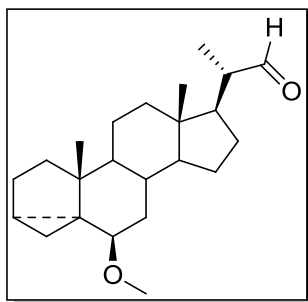


i-Stigmasterol methyl ether **82** (0.500 g, 1.17 mM) was dissolved in dichloromethane (37 mL) in a 100 mL Shlenck tube. The inlet of the Shlenck tube was connected, *via* rubber tubing, to an ozone generator. The outlet was connected in series to two dreschel bottles containing 10% potassium iodide in water solution (100 mL and 50 mL respectively). The solution was cooled to $-78\text{ }^{\circ}\text{C}$ using acetone and liquid nitrogen. A stream of oxygen was allowed to pass through the apparatus for 5 min to ensure there were no leakages. The ozone generator was switched on and ozone was allowed to pass through the substrate for 40 min. Upon reaction completion, excess ozone was removed by passing a stream of oxygen through the apparatus for 5 min. Excess triphenylphosphine was added to the Shlenck tube and was allowed to stir with the reaction solution overnight. An aliquot of the reaction solution was concentrated under reduced pressure and ^1H NMR analysis was obtained on this material. The ^1H NMR spectrum of the crude product showed only peaks in the aromatic region, owing to the vast excess of triphenyl phosphine oxide present. The remainder of the reaction solution was transferred onto celite, and concentrated under reduced pressure. Purification required repeated column chromatography using gradient hexane to 98:2 hexane : ethyl acetate, in order to remove excess triphenylphosphine oxide, and yielded the desired product **85** as a white solid (0.314 g, 54%): $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 2930, 1726; δ_{H} (300 MHz, CDCl_3) 0.43 (1H, dd, J 8.0, 5.1), 0.63 – 0.68 (1H, m), 0.77 (3H, s, 18-CH₃), 0.82 – 0.98 (7H, m), 1.02 – 2.05 [19H, m, containing 1.03 (3H, s, 19-CH₃) and 1.13 (3H, d, J 6.8, 21-CH₃)], 2.32 – 2.45 (1H, m, 20-CH), 2.78 (1H, t, J 2.8, 6-CH), 3.33 (3H, s, OMe, CH₃), 9.58 (1H, d, J 3.2, 22-CH); δ_{C} (75 MHz, CDCl_3) 12.6 (CH₃), 13.1 (CH₂), 13.4 (CH₃), 19.3 (CH₃), 21.5 (CH), 22.7 (CH₂), 24.6 (CH₂), 24.9 (CH₂), 27.1 (CH₂), 30.5 (CH), 33.4 (CH₂), 35.1 (CH₂), 40.0 (CH₂), 43.39 (quaternary C), 43.41 (quaternary C), 48.0 (CH), 49.5 (CH), 51.2 (CH), 55.8 (CH), 56.6 (OMe, CH₃), 82.3 (CH), 205.3 (22-CH).

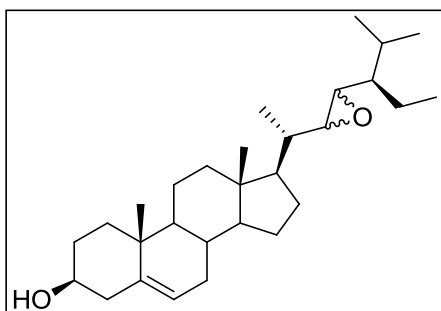
i*-Stigmasterol methyl ether 22,23 epoxide³² **84a** and **84b*

i-Stigmasterol methyl ether **82** (0.255 g, 0.60 mM) was dissolved in dichloromethane (150 mL) and *m*-CPBA (0.103 g, 0.60 mM) was added at 0 °C. The reaction solution was allowed to warm to room temperature and stirred for 6 h. The reaction mixture was filtered and washed with 10% sodium bicarbonate (4 × 50 mL) and brine (1 × 50 mL). The organic solution was dried over magnesium sulfate and

concentrated under reduced pressure to yield the crude product (0.270 g) as a pale yellow solid. Purification by column chromatography using hexane : ethyl acetate (95:5) yielded the desired compounds **84a** and **84b** as a white solid (0.175 g, 66%). The analysis described below pertains to the mixture of α and β epoxides in a 1:1 ratio, which were inseparable: mp 184 – 185 °C (literature,³² 186 – 188); δ_H (300 MHz, CDCl₃) 0.40 – 0.47 (2H, m, 3-CH for both diastereomers), 0.61 – 0.68 (2H, m), 0.715 and 0.722 (3H, s, 18-CH₃ of α and β diastereomers), 0.75 – 2.03 [78H, m, containing 0.93 (6H, d, *J* 6.9, 26-CH₃ and 27-CH₃), 1.02 (3H, s, 19-CH₃), 21-CH₃ and 29-CH₃ for both diastereoisomers], 2.43 – 2.54 (3H, m, 22-CH₂, 22-CH₃, 23-CH of one diastereomer), 2.70 – 2.80 (3H, m, 23-CH of one diastereomer, 6-CH for both diastereomers), 3.32 (3H, s, OMe, CH₃ of one diastereomer), 3.33 (3H, s, OMe, CH₃ of one diastereomer); δ_C (75 MHz, CDCl₃) 12.24 (CH₃), 12.38 (CH₃), 12.46 (CH₃), 16.13 (CH₃), 16.27 (CH₃), 19.27 (CH₃), 19.31 (CH₃), 19.36 (CH₃), 19.44 (CH₃), 19.60 (CH₃), 20.18 (CH₃), 20.89 (CH₂), 20.94 (CH₂), 21.44 (CH), 21.56 (CH), 22.72 (CH₂), 22.77 (CH₂), 24.43 (CH₂), 24.44 (CH₂), 24.95 (CH₂), 24.98 (CH₂), 27.20 (CH₂), 28.02 (CH₂), 29.14 (CH), 29.32 (CH), 30.47 (CH), 30.52 (CH), 33.35 (CH₂), 33.36 (CH₂), 34.94 (CH₂), 35.12 (CH₂), 35.20 (quaternary C), 35.36 (quaternary C), 38.64 (CH), 38.92 (CH), 40.06 (CH₂), 40.18 (CH₂), 43.08 (quaternary C), 43.13 (quaternary C), 43.38 (quaternary C), 43.39 (quaternary C), 48.01 (CH), 48.07 (CH), 48.30 (CH), 48.79 (CH), 53.60 (CH), 56.09 (CH), 56.14 (CH), 56.20 (CH), 56.57 (OMe, CH₃ of one diastereomer), 56.59 (OMe, CH₃ of one diastereomer), 58.55 (23-CH of one diastereomer), 62.07 (CH), 62.17 (23-CH of one diastereomer), 63.18 (22-CH for both diastereomers), 82.36 (6-CH of one diastereomer), 82.37 (6-CH of one diastereomer); exact mass calculated for C₃₀H₅₁O₂ [M + H]⁺ 443.3889, found 443.3870.

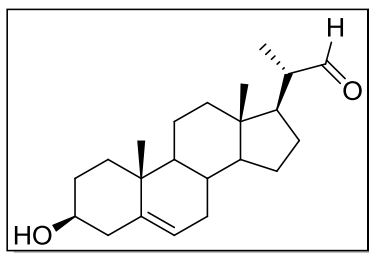
Attempted synthesis of 20(*S*)-methyl-6(*R*)-methoxy-3,5-cyclo-pregnane-21-al **85**

Periodic acid (0.044 g, 0.19 mM) was added to a stirring solution of epoxides **84a** and **84b** (0.030 g, 0.07 mM) in diethyl ether (20 mL). The resulting solution was stirred for 3 h at 25 °C and monitored by TLC analysis. Upon reaction completion, the solution was cooled to room temperature and water (20 mL) was added. The organic layer was separated and washed with water (2 × 50 mL) and brine (1 × 50 mL). The organic component was dried over magnesium sulfate and concentrated under reduced pressure¹² to yield the crude product as an off white solid (0.035 g). Analysis of the ¹H NMR spectrum of the crude product showed no evidence of the formation of the desired aldehyde. Purification by column chromatography using gradient elution 90:10 to 80:20 hexane : ethyl acetate led to the isolation of **76a** and **76b** as a white solid in a 1:1 ratio (0.020 g, 67%). ¹H NMR analysis described below:

22,23-Epoxy-stigmasterol³² **76a and **76b****

CH).

δ_H (300 MHz, CDCl₃) 0.69 (3H, s, 18-CH₃), 0.80 – 1.73 [38H, m, containing 21-CH₃, 19-CH₃, 26-CH₃, 27-CH₃, and 29-CH₃], 1.76 – 2.11 (6H, m), 2.19 – 2.37 (2H, m), 2.46 – 2.54 (1H, m, one of 22-CH *R* or *S*, or 23-CH *R* or *S*), 2.74 (1H, dd, *J* 7.1, 2.3, one of 22-CH *R* or *S*, or 23-CH *R* or *S*), 3.52 (1H br s, 3-CH), 5.35 (1H, d, *J* 5.2, 6-

3 β -Hydroxy-20(*S*)-methyl-pregnan-5-ene-21-al³⁴ **75**

Periodic acid (0.190 g, 0.83 mM) was added to a stirring solution of aldehyde **85** (0.100 g, 0.29 mM) in diethyl ether (50 mL). The resulting solution was stirred for 3 h at 25 °C and monitored by TLC analysis. Upon reaction completion, the solution was cooled to room temperature and water (20 mL) was added. The organic layer was separated and washed with water (2 × 50 mL) and brine (1 × 50 mL). The organic component was dried over magnesium sulfate and concentrated under reduced pressure to yield the crude product **75** as an off white solid (0.076

g, 79%); mp 140 – 145 °C (literature,³⁴ 142 – 143 °C); $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3435, 2925, 1720; δ_{H} (300 MHz, CDCl_3) 0.60 – 2.45 [31H, m, containing (0.73, 3H, s, 18- CH_3), 1.02 (3H, s, 19- CH_3), 1.13 (3H, d, J 6.9, 21- CH_3) and 20-CH], 3.46 – 3.59 (1H, m, 3-CH), 5.35 (1H, d, J 5.3, 6-CH), 9.57 (1H, d, J 3.3, 22-CH); δ_{C} (75 MHz, CDCl_3) 11.2 (CH_3), 12.4 (CH_3), 18.4 (CH_3), 20.0 (CH_2), 23.6 (CH_2), 26.0 (CH_2), 28.7 (CH_2), 30.6 (CH_2), 30.8 (CH_2), 30.9 (CH), 30.9 (quaternary C), 35.5 (quaternary C), 36.2 (CH_2), 38.5 (CH_2), 41.3 (CH_2), 48.5 (CH), 49.1 (CH), 50.0 (CH), 55.0 (CH), 70.7 (3-CH), 120.5 (6-CH), 139.8 (5-quaternary C), 204.1 (24-CH); exact mass calculated for $\text{C}_{22}\text{H}_{35}\text{O}_2$ $[\text{M} + \text{H}]^+$ 331.2637, found 331.2626.

Method B:

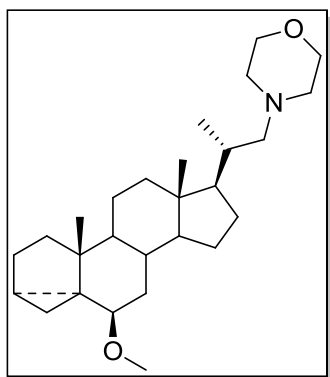
Epoxide **76** (0.018 g, 0.02 mM) was stirred in diethyl ether (10 mL). Periodic acid (0.027 g, 0.12 mM) was added and the reaction mixture was stirred at a gentle reflux for 6 h and room temperature for a further 16 h. Water (20 mL) was added. The organic layer was separated and washed with water (2×50 mL) and brine (1×50 mL). The organic component was dried over magnesium sulfate and concentrated under reduced pressure¹² to yield the crude product as an off white oily solid (0.016 g). Analysis of the ^1H NMR spectrum of the crude material showed no indication of formation of the desired aldehyde **75**, and only starting material **76** was evident.

Method C³⁵:

Sodium periodate (0.016 g, 0.07 mM) was stirred for 5 min in a 2:1 mixture of tetrahydrofuran and water. Epoxide **76** (0.016 g, 0.04 mM) was added and the reaction was stirred at room temperature until the starting material appeared to have been consumed, as indicated by TLC analysis. The reaction mixture was filtered and washed with diethyl ether (20 mL). The water layer was separated and washed with diethyl ether (2×10 mL). The combined ether extracts were washed with water (2×20 mL), followed by brine (20 mL). The organic layer was dried over magnesium sulfate and concentrated under reduced pressure to yield an off white oily solid (0.010 g). Inspection of the ^1H NMR spectrum of the crude material showed peaks characteristic of the starting material **76**. The aqueous layer was concentrated under reduced pressure, however, no evidence of product formation was present. The aqueous layer was then extracted with dichloromethane and chloroform respectively, with no evidence of product formation.

Method D³⁵:

Sodium periodate (0.017 g, 0.08 mM) was stirred for 5 min in a 4:1 mixture of acetonitrile and water. Epoxide **76** (0.009 g, 0.02 mM) was added and the reaction was stirred at room temperature for 6 h. The reaction mixture was filtered and washed with diethyl ether (10 mL). The water layer was separated and washed with diethyl ether (2×5 mL). The combined ether extracts were washed with water (2×10 mL), followed by brine (10 mL). The organic layer was dried over magnesium sulfate and concentrated under reduced pressure to yield an off white oily solid (0.006 g). Inspection of the ^1H NMR spectrum of the crude material showed peaks characteristic of the starting material **76**, with no evidence of product formation.

20(*S*)-Methyl-6(*R*)-methoxy-3,5-cyclo-21-morpholio-pregnane **86**

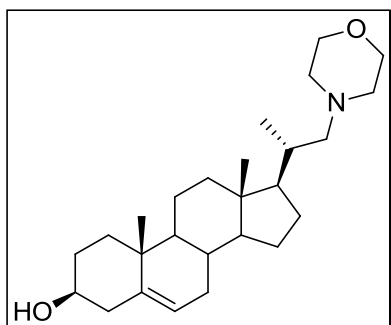
Sodium triacetoxyborohydride (0.022 g, 0.07 mM) was added to a solution of 20(*S*)-methyl-6(*R*)-methoxy-3,5-cyclo-pregnane-21-al **85** (0.025 g, 0.10 mM) and morpholine (6 μL , 0.07 mM) in 1,2-dichloroethane (10 mL). The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate solution (10 mL) was added. The reaction mixture was extracted into ethyl acetate (10 mL) and washed with brine (2×10 mL). The organic extracts were then dried

over magnesium sulfate and concentrated under reduced pressure to yield the crude product as an off white solid with 60% conversion. The product was purified by column chromatography using 85:15 ethyl acetate : ether to yield the desired product **86** as a white solid (0.013 g, 43%): mp 162 – 164 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 2930, 1119, 1099; δ_{H} (300 MHz, CDCl_3) 0.43 (1H dd, J 8.0, 5.1, 3-CH), 0.61 – 0.67 (1H, m), 0.74 (3H, s, 18- CH_3), 0.78 – 2.10 [40H, m, containing 1.00 (3H, d, J 6.5, 21- CH_3), 1.02 (3H, s, 19- CH_3), 2.02 (1H, dt, J 12.5, 3.0, 1 hydrogen of 22- CH_2)], 2.14 – 2.32 (3H, m, containing 2 hydrogens of N- CH_2 and 1 hydrogen of 22- CH_2), 2.42 – 2.55 (2H, m, 2 hydrogens of N- CH_2), 2.77 (1H, t J 2.7, 6-CH), 3.33 (3H, s, OMe, CH_3), 3.60 – 3.77 (4H, m, morpholine O- $\text{CH}_2 \times 2$); δ_{C} (75 MHz, CDCl_3) 12.4 (CH_3), 13.1 (CH_2), 17.9 (CH_3), 19.3 (CH_3), 21.5 (CH), 22.8 (CH_2), 24.3 (CH_2), 25.0 (CH_2), 28.3 (CH_2), 29.7 (CH_2), 30.5 (CH), 33.4 (CH_2), 33.8 (CH), 35.1 (CH_2), 40.2 (CH_2), 43.2 (quaternary C), 43.4 (quaternary C), 48.1 (CH), 54.3 (CH_2), 55.4 (CH), 56.3 (CH), 56.6

(OMe, CH₃), 65.2 (CH₂), 67.2 (CH₂), 82.4 (CH); exact mass calculated for C₂₇H₄₆NO₂ [M + H]⁺ 416.3529, found 416.3535.

For reductive aminations in this section: Procedure as per Abdel-Magid reference.²⁵ Assignments based on Taniguchi paper.²⁸

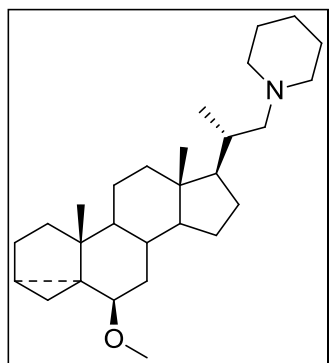
Attempted synthesis of 3 β -hydroxy-20(*S*)-methyl-21-morpholino-pregnane-5-ene **94 by deprotection of 20(*S*)-methyl-6(*R*)-methoxy-3,5-cyclo-21-morpholio-pregnane **86****



Periodic acid (0.008 g, 0.03 mM) was added to a stirring solution of 20(*S*)-methyl-6(*R*)-methoxy-3,5-cyclo-21-morpholio-pregnane **86** (0.010 g, 0.02 mM) in diethyl ether (10 mL). The resulting solution was stirred for 3 h at 25 °C and monitored by TLC analysis. Upon consumption of the starting material, the solution was cooled to room temperature and water (10 mL) was added. The organic

layer was separated and washed with water (2 × 20 mL) and brine (1 × 20 mL). The organic component was dried over magnesium sulfate and concentrated under reduced pressure to yield the crude product (0.007 g). The ¹H NMR spectrum of the crude material showed no evidence of formation of the desired product **94**. The aqueous layer from the work up was extracted into ethyl acetate and concentrated under reduced pressure. Again the ¹H NMR spectrum of the crude material showed no evidence of formation of the desired product.

20(*S*)-Methyl-6(*R*)-methoxy-3,5-cyclo-21-piperidino-pregnane **87**

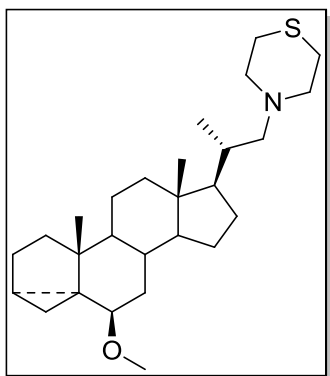


Sodium triacetoxyborohydride (0.074 g, 0.35 mM) was added to a solution of 20(*S*)-methyl-6(*R*)-methoxy-3,5-cyclo-pregnane-21-al **85** (0.086 g, 0.25 mM) and piperidine (25 μ L, 0.25 mM) in 1,2-dichloroethane (20 mL). The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate solution (20 mL) was added. The reaction mixture was extracted into ethyl acetate (20 mL) and washed

with brine (2 × 20 mL). The organic extracts were then dried over magnesium sulfate and

concentrated under reduced pressure to yield the crude product as a dark orange solid with 50% conversion. The product was purified by column chromatography using 85:15 ethyl acetate : ether to yield the desired product **87** as an orange solid (0.019 g, 13%): mp 167 – 168 °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 2932; δ_{H} (300 MHz, CDCl_3) 0.43 (1H, dd, J 8.0, 5.1, 3-CH), 0.61 – 0.67 (1H, m), 0.70 – 2.06 [39H, m, containing 0.73 (3H, s, 18-CH₃), 1.00 (3H, d, J 6.3, 21-CH₃), 1.02 (19-CH₃), 2.01 (2H, dt, J 7.1, 3.0, 22-CH₂ 1 hydrogen of 22-CH₂); piperidine (CH₂ × 3)], 2.09 – 2.28 (3H, m, containing 2 hydrogens of N-CH₂ and 1 hydrogen of 22-CH₂), 2.30 – 2.53 (2H, m, 2 hydrogens of N-CH₂), 2.77 (1H, t, J 2.70, 6-CH), 3.33 (3H, s, OMe, CH₃); δ_{C} (75 MHz, CDCl_3) 12.4 (CH₃), 13.1 (CH₂), 18.1 (CH₃), 19.3 (CH₃), 21.5 (CH), 22.8 (CH₂), 24.4 (CH₂), 25.0 (CH₂), 28.3 (CH₂), 30.5 (CH), 33.4 (CH₂), 35.1 (CH₂), 40.2 (CH₂), 48.0 (CH), 56.3 (CH), 56.6 (OMe, CH₃), 82.4 (CH); exact mass calculated for $\text{C}_{28}\text{H}_{48}\text{NO}$ $[\text{M} + \text{H}]^+$ 414.3736, found 414.3729.

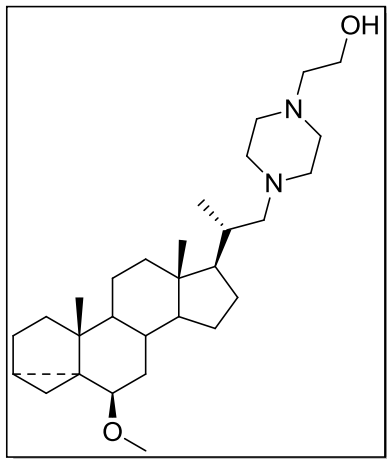
20(*S*)-Methyl-6(*R*)-methoxy-3,5-cyclo-21-thiomorpholino-pregnane **88**



Sodium triacetoxyborohydride (0.086 g, 0.41 mM) was added to a solution of 20(*S*)-methyl-6(*R*)-methoxy-3,5-cyclo-pregnane-21-al **85** (0.100 g, 0.29 mM) and thiomorpholine (30 μL , 0.29 mM) in 1,2-dichloroethane (50 mL). The reaction mixture was stirred at room temperature for 1.5 h, after which time saturated aqueous sodium bicarbonate solution (50 mL) was added. The reaction mixture was extracted into ethyl acetate (50 mL) and washed with brine (2 × 50 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield the crude product as an off white solid with 80% conversion. Purification required repeated column chromatography using 85:15 ethyl acetate : ether, yielding the desired product **88** as a yellow solid (0.051 g, 29%) mp 158 – 160 °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 2926; δ_{H} (300 MHz, CDCl_3) 0.43 (1H, dd, J 8.0, 5.0, 3-CH), 0.64 – 0.70 (1H, m), 0.71 – 1.95 [29H, m, containing 0.73 (3H, s, 18-CH₃), 0.97 (3H, d, J 6.4, 21-CH₃), 1.02 (3H, s, 19-CH₃)], 2.01 (1H, dt, J 11.8, 2.8, 1 hydrogen of 22-CH₂), 2.31 (1H, dd, J 12.4, 3.2, 1 hydrogen of 22-CH₂), 2.40 – 2.72 (9H, m, containing 6-CH and thiomorpholine CH₂ × 4), 3.33 (3H, s, OMe, CH₃); δ_{C} (75 MHz, CDCl_3) 12.4 (CH₃), 18.0 (CH₃), 19.3 (CH₃), 21.5 (CH), 22.7 (CH₂), 24.4 (CH₂), 25.0 (CH₂), 28.1 (CH₂), 28.3 (CH₂), 29.7 (CH₂), 30.5 (CH), 34.2 (CH), 35.1 (CH₂), 40.2 (CH₂), 43.1 (quaternary C), 43.4 (quaternary C), 48.0 (CH), 55.4

(CH), 55.7 (CH₂), 56.3 (CH), 56.6 (OMe, CH₃), 65.4 (CH₂), 82.3 (CH); m/z (ESI⁺) 432 [M + H]⁺ (87%) 400 (100); exact mass calculated for C₂₇H₄₆NOS [M + H]⁺ 432.330, found 432.3318.

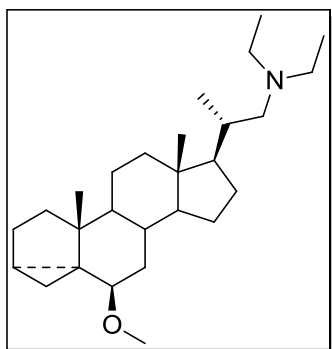
20(*S*)-Methyl-6(*R*)-methoxy-3,5-cyclo-21-(*N*-2-hydroxyethyl-piperazino)-pregnane **89**



Sodium triacetoxyborohydride (0.047 g, 0.22 mM) was added to a solution of 20(*S*)-methyl-6(*R*)-methoxy-3,5-cyclo-pregnane-21-al **85** (0.055 g, 0.16 mM) and 1-(2-hydroxyethyl piperazine) (0.020 g, 18 μ L, 0.16 mM) in 1,2-dichloroethane (30 mL). The reagent was weighed in the following manner: a drop of 1-(2-hydroxyethyl)piperazine was placed in a vial and its mass was recorded as 0.100 g. 10 mL of the reaction solvent was added and the amine was allowed to go into solution. 2 mL of this resulting solution

was measured by syringe and transferred into the reaction vessel. The reaction mixture was stirred at room temperature for 24 h, and monitored by TLC analysis. After this time, saturated aqueous sodium bicarbonate solution (30 mL) was added. The reaction mixture was extracted into ethyl acetate (30 mL) and washed with brine (2 \times 30 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield the desired product **89** as an off white solid with \geq 95% conversion (0.023 g, 31% isolated yield) mp 166 – 169 °C; ν_{max} (KBr)/cm⁻¹ 2930, 1099; δ_{H} (300 MHz, CDCl₃) 0.43 (1H, dd, J 8.0, 5.1, 3-CH), 0.64 – 0.70 (1H, m), 0.73 (3H, s, 18-CH₃), 0.77 – 2.08 [27H, m, containing 0.99 (3H, d, J 6.4, 21-CH₃), 1.02 (3H, s, 19-CH₃) and one hydrogen of 22-CH₂], 2.10 – 2.40 (5H, m, containing N-CH₂ \times 2 and one hydrogen of 22-CH₂), 2.45 – 2.70 (6H, m containing N-CH₂ \times 3), 2.77 (1H, t, J 2.7, 6-CH), 3.33 (3H, s, OMe, CH₃), 3.61 (2H, t, J 5.3, CH₂-OH); δ_{C} (75 MHz, CDCl₃) 12.4 (CH₃), 13.1 (CH₂), 18.0 (CH₃), 19.3 (CH₃), 21.5 (CH), 22.8 (CH₂), 24.4 (CH₂), 25.0 (CH₂), 28.3 (CH₂), 29.7 (CH₂), 30.5 (CH), 33.4 (CH₂), 34.1 (CH), 35.1 (CH₂), 40.2 (CH₂), 43.1 (quaternary C), 43.4 (quaternary C), 48.1 (CH), 53.0 (CH₂), 55.5 (CH), 56.3 (CH), 56.6 (CH₃), 57.6 (CH₂), 59.2 (CH₂), 64.7 (CH₂), 82.4 (CH); m/z (ESI⁺) 459 [M + H]⁺ (100%); exact mass calculated for C₂₉H₅₁N₂O₂ [M + H]⁺ 459.3951, found 459.3938.

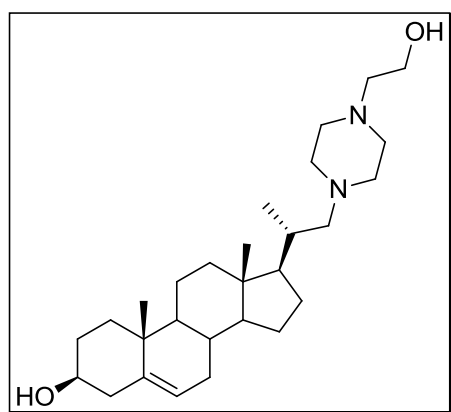
Attempted Synthesis of 20(*S*)-Methyl-6(*R*)-methoxy-3,5-cyclo-21-diethylamino-pregnane **90**



Sodium triacetoxyborohydride (0.051 g, 0.24 mM) was added to a solution of 20(*S*)-methyl-6(*R*)-methoxy-3,5-cyclo-pregnane-21-al **85** (0.060 g, 0.17 mM) and diethylamine (18 μ L, 0.17 mM) in 1,2-dichloroethane (30 mL). The reaction mixture was stirred at room temperature for 1.5 h. After this time, saturated aqueous sodium bicarbonate solution (30 mL) was added. The reaction mixture was extracted into ethyl acetate (30 mL) and washed with brine (2×30 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield the crude product. Analysis of the ^1H NMR spectrum of the crude material showed only starting aldehyde **85** was present, with no evidence of formation of the desired product **90**.

The reaction was repeated using the crude material from the first attempt. Identical reaction conditions were employed, with the reaction time being increased to 24 h. Again, there was no evidence of formation of the desired product by analysis of the ^1H NMR spectrum of the crude reaction material.

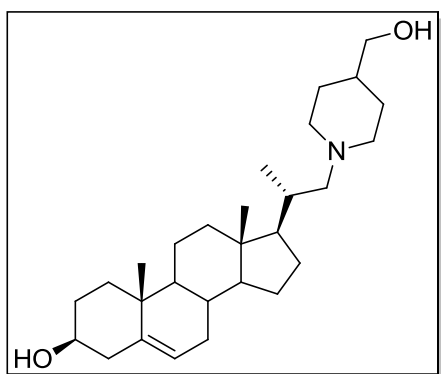
3 β -Hydroxy-20(*S*)-methyl-21-piperazino-*N*-2-hydroxyethyl-pregnan-5-ene **91**



Sodium triacetoxyborohydride (0.047 g, 0.22 mM) was added to a solution of 3 β -hydroxy-20(*S*)-methyl-pregnan-5-ene-21-al **75** (0.052 g, 0.16 mM) and 1-(2-hydroxyethyl piperazine) (0.020 g, 19 μ L, 0.16 mM) in 1,2-dichloroethane (30 mL). The reagent was weighed in the following manner: a drop of 1-(2-hydroxyethyl)piperazine was placed in a vial and its mass was recorded as 0.100 g. 10 mL of the reaction solvent was added and the amine was allowed to go into solution. 2 mL of this resulting solution was measured by syringe and transferred into the reaction vessel. The reaction mixture was stirred at room temperature for 24 h, and monitored by TLC analysis. After this time, saturated aqueous sodium bicarbonate solution (30 mL) was added. The reaction

mixture was extracted into ethyl acetate (30 mL) and washed with brine (2×30 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield the desired product **91** as a light yellow solid with $\geq 95\%$ conversion (0.019 g, 24% isolated yield): mp 162 – 166 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3432, 2962, 1261; δ_{H} (300 MHz, CDCl_3) 0.60 – 2.40 [38H, m, containing (0.70, 3H, s, 18- CH_3), 1.01 (3H, s, 19- CH_3), 21- CH_3], 2.45 – 2.73 (6H, m, containing piperazine $\text{N-CH}_2 \times 3$), 3.46 – 3.59 (1H, m, 3-CH), 3.63 (1H, t, J 5.2, $\text{CH}_2\text{-OH}$), 5.35 (1H, apparent d, J 5.2, 6-CH); δ_{C} (75 MHz, CDCl_3) 11.9 (CH_3), 18.0 (CH_3), 19.4 (CH_3), 21.0 (CH_2), 24.4 (CH_2), 28.2 (CH_2), 29.7 (CH_2), 31.6 (CH_2), 31.9 (CH), 32.0 (CH_2), 34.1 (CH), 37.2 (CH_2), 39.7 (CH_2), 42.3 (CH_2), 42.3 (quaternary C), 42.7 (quaternary C), 50.1 (CH), 52.9 (CH_2), 55.2 (CH), 56.5 (CH), 57.5 (CH_2), 59.3 (CH_2), 64.6 (CH_2), 71.8 (CH), 121.6 (CH), 140.8 (quaternary C); m/z (ESI^+) 445 [$\text{M} + \text{H}$] $^+$ (98%), 490 (100); exact mass calculated for $\text{C}_{28}\text{H}_{49}\text{N}_2\text{O}_2$ [$\text{M} + \text{H}$] $^+$ 445.3794, found 445.3801.

Attempted synthesis of 3 β -Hydroxy-20(S)-methyl-21-(4-hydroxymethylpiperidino)-pregnan-5-ene **92**



Sodium triacetoxyborohydride (0.039 g, 0.18 mM) was added to a solution of 3 β -hydroxy-20(S)-methylpregnan-5-ene-21-al **75** (0.045 g, 0.13 mM) and 4-piperidine methanol (0.015 g, 0.13 mM) in 1,2-dichloroethane (20 mL). The reaction mixture was stirred at room temperature for 1.5 h. After this time, saturated aqueous sodium bicarbonate solution (20 mL)

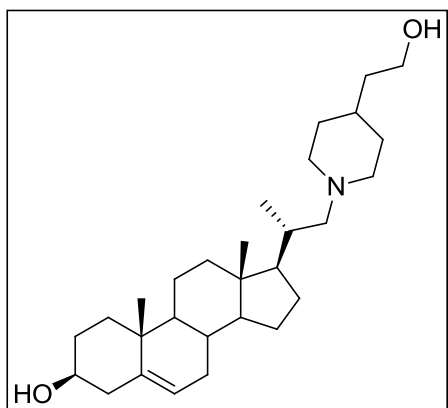
was added. The reaction mixture was extracted into ethyl acetate (20 mL) and washed with brine (2×20 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield the crude product. Analysis of the ^1H NMR spectrum of the crude material showed ~40% starting aldehyde remained, with new multiplets at 2.75 – 2.85 and 2.86 – 3.00 ppm integrating for ~30% each.

The reaction was repeated using the crude product from the previous attempt. Reaction conditions were identical. In this case, 20% aldehyde remained, with the new multiplets increasing to ~60% each.

The reaction was repeated using the crude product from the previous attempt. Reaction conditions were identical. In this case, 5% aldehyde remained, with the new multiplets increasing by 5% to ~65% each.

The reaction was not pursued further.

Attempted synthesis of 3 β -hydroxy-20(*S*)-methyl-21-(4-hydroxyethylpiperidino)-pregnan-5-ene **93**



Sodium triacetoxyborohydride (0.017 g, 0.08 mM) was added to a solution of 3 β -hydroxy-20(*S*)-methylpregnan-5-ene-21-al **75** (0.020 g, 0.06 mM) and 4-piperidine ethanol (0.008 g, 0.06 mM) in 1,2-dichloroethane (10 mL). The reaction mixture was stirred at room temperature for 1.5 h. After this time, saturated aqueous sodium bicarbonate solution (10 mL) was added. The reaction mixture was extracted into ethyl acetate (10 mL) and washed with brine (2 \times 10 mL). The organic extracts were then dried over magnesium sulfate and concentrated under reduced pressure to yield the crude product (0.011 g). Analysis of the ^1H NMR spectrum of the crude material showed ~70% starting aldehyde remained, with no evidence of formation of the desired product **93**. The reaction was not repeated due to the limited quantity of material.

3.5 References

- Kavtaradze, L. K.; Manley-Harris, M.; Nicholson, B. K. *Steroids* **2004**, *69*, 697-700.
- Emmons, G. T.; Wilson, W. K.; Schroepfer, G. J. *Magn. Reson. Chem.* **1989**, *27*, 1012-1024.
- Male, K. B.; Crowley, S. M.; Collins, S. G.; Tzeng, Y. M.; Luong, J. H. T. *Anal. Methods* **2010**, *2*, 870-877.
- Mazzocchi, P. H.; Wilson, P.; Khachik, F.; Klingler, L.; Minamikawa, S. *J. Org. Chem.* **1983**, *48*, 2981-2989.
- McCarthy, F. O.; Chopra, J.; Ford, A.; Hogan, S. A.; Kerry, J. P.; O'Brien, N. M.; Ryan, E.; Maguire, A. R. *Org. Biomol. Chem.* **2005**, *3*, 3059-3065.
- Shingate, B. B.; Hazra, B. G.; Salunke, D. B.; Pore, V. S. *Tetrahedron Lett.* **2011**, *52*, 6007-6010.
- Zhao, Y. J.; Loh, T. P. *Tetrahedron* **2008**, *64*, 4972-4978.
- Emmons, G. T.; Wilson, W. K.; Schroepfer, G. J. *J. Lipid Res.* **1989**, *30*, 133-138.
- Chung, S. K.; Ryoo, C. H.; Yang, H. W.; Shim, J. Y.; Kang, M. G.; Lee, K. W.; Kang, H. I. *Tetrahedron* **1998**, *54*, 15899-15914.
- Shimizu, N.; Itoh, T.; Saito, M.; Matsumoto, T. *J. Org. Chem.* **1984**, *49*, 709-712.
- Sonoda, Y.; Sato, Y. *Chem. Pharm. Bull.* **1983**, *31*, 1698-1701.
- Barton, D. H. R.; Mellows, G.; Widdowso, D. A.; Wright, J. J. *J. Chem. Soc. C-Org.* **1971**, 1142.
- Parish, E. J.; Sun, H.; Kizito, S.; Boos, T. L. *Molecules* **2000**, *5*, 114-117.
- Kavtaradze, L. K.; Manley-Harris, M.; Nicholson, B. K. *Steroids* **2004**, *69*, 227-233.
- Mori, N.; Togo, H. *Tetrahedron* **2005**, *61*, 5915-5925.
- Haddad, Y. M. Y.; Henbest, H. B.; Husbands, J.; Mitchell, T. R.; Trochagr, J. *J. Chem. Soc. Perk. T 1* **1974**, 596-601.
- Kennedy, E. M.; P'Pool, S. J.; Jiang, J. H.; Sliva, D.; Minto, R. E. *J. Nat. Prod.* **2011**, *74*, 2332-2337.
- Irvine, D. S.; Lawrie, W.; McNab, A. S.; Spring, F. S. *J. Chem. Soc.* **1956**, 2029-2033.
- Brewis, S.; Sayer, G. C.; Halsall, T. G. *J. Chem. Soc.* **1962**, 2763-2766.
- Chaudhur, N. K.; Williams, J. G.; Nickolso, R.; Gut, M. *J. Org. Chem.* **1969**, *34*, 3759.
- Paryzek, Z.; Martynow, J. *Can. J. Chem.* **1988**, *66*, 2130-2136.
- Ruzicka, L.; Montavon, M.; Jeger, O. *Helv. Chim. Acta* **1948**, *31*, 818-821.
- Bancroft, G.; Haddad, Y. M. Y.; Summers, G. H. R. *J. Chem. Soc.* **1961**, 3295-3303.
- Ellis, G.; Jones, R. G. *J. Chem. Soc. Perk. T 2* **1972**, 437-440.
- AbdelMagid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849-3862.
- Lavrado, J.; Cabal, G. G.; Prudencio, M.; Mota, M. M.; Gut, J.; Rosenthal, P. J.; Diaz, C.; Guedes, R. C.; dos Santos, D. J. V. A.; Bichenkova, E.; Douglas, K. T.; Moreira, R.; Paulo, A. *J. Med. Chem.* **2011**, *54*, 734-750.
- Csuk, R.; Barthel, A.; Raschke, C.; Kluge, R.; Strohl, D.; Trieschmann, L.; Bohm, G. *Arch. Pharm.* **2009**, *342*, 699-709.
- Taniguchi, Y.; Horie, S.; Takaki, K.; Fujiwara, Y. *J. Organomet. Chem.* **1995**, *504*, 137-141.
- Zhang, C. L.; Tan, C. Y.; Zu, X. Y.; Zhai, X.; Liu, F.; Chu, B. Z.; Ma, X. H.; Chen, Y. Z.; Gong, P.; Jiang, Y. Y. *Eur. J. Med. Chem.* **2011**, *46*, 1404-1414.
- Lee, Y. S.; Rho, E. S.; Min, Y. K.; Kim, B. T.; Kim, K. H. *J. Carbohydr. Chem.* **2001**, *20*, 503-506.
- Pellissier, H. *Tetrahedron* **2004**, *60*, 5123-5162.
- Misharin, A. Y.; Mehtiev, A. R.; Morozovich, G. E.; Tkachev, Y. V.; Timofeev, V. P. *Bioorg. Med. Chem.* **2008**, *16*, 1460-1473.
- Foley, D. A.; O'Callaghan, Y.; O'Brien, N. M.; McCarthy, F. O.; Maguire, A. R. *J. Agr. Food. Chem.* **2010**, *58*, 1165-1173.
- Cui, J. G.; Lin, C. W.; Zeng, L. M.; Su, J. Y. *Steroids* **2002**, *67*, 1015-1019.
- Telvekar, V.; Patel, D.; Mishra, S. *Synthetic Commun.* **2009**, *39*, 311-315.

36. O'Connell, N. M.; O'Callaghan, Y. C.; O'Brien, N. M.; Maguire, A. R.; McCarthy, F. O. *Tetrahedron* **2012**, *68*, 4995-5004.
37. Antonchick, A. P.; Schneider, B.; Zhabinskii, V. N.; Khripach, V. A. *Steroids* **2004**, *69*, 617-628.

Appendix

Abbreviations / Definitions



Appendix

[O]: Oxidation

°C: degrees Celsius

A. camphorata: *Antrodia camphorata*

Å: angstrom

Aq: aqueous

Ar: aryl

br, s: broad singlet

CaCo-2: human colon cancer cell line

CDCl₃: deuterated chloroform

Cr^{III}: Chromium in the +3 oxidation state

Cr^{VI}: Chromium in the +6 oxidation state

d: doublet

DCE: dichloroethane

DCM: dichloromethane

dd: doublet of doublets

DDQ: 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone

DEPT: Distortionless Enhancement by Polarisation Transfer

DMAP: 4-dimethylaminopyridine

DMF: *N,N*-Dimethylformamide

DNA: deoxyribonucleic acid

E. coli: *Escherichia coli*

ECIS₅₀: half inhibitory concentration measured using Electric Cell Impedance Sensing

ED₅₀: effective dose for 50% of a population base

eq: equivalent

ESI: electrospray ionisation

FDA/EtBr: Fluorescein Diacetate/Ethidium Bromide (A biological assay)

g: gram

h: hour

HRMS: High Resolution Mass Spectrometry

Hz: Hertz

IC₅₀: half maximal inhibitory concentration

IR: infrared

LDL: low density lipoprotein

LOP: Lanosterol Oxidation Product

m: multiplet

m-CPBA: meta-chloroperoxybenzoic acid

mg: milligram

MHz: megahertz

min: minute

mL: millilitre

mM: millimole

mp: melting point

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (A biological assay)

NAD⁺: nicotinamide adenine dinucleotide

NADH: the reduced form of nicotinamide adenine dinucleotide

NADP⁺: nicotinamide adenine dinucleotide phosphate

NADPH: the reduced form of nicotinamide adenine dinucleotide phosphate

nM: nanomolar

NMO: *N*-methylmorpholine *N*-oxide

NMR: Nuclear Magnetic Resonance

p: para

Pd/C: palladium on carbon

ppm: parts per million

psi: pound per square inch

r.t: room temperature

R_f: retardation factor

s: singlet

Sf9: *Spodoptera frugiperda* insect cells

SM: Starting Material

t: triplet

THF: tetrahydrofuran

TLC: Thin Layer Chromatography

TMS: tetramethylsilane

Ts: tosyl

U937: human lymphoma cell line

US: United States

Δ: heat

Appendix

Δ^n : Position of the alkene bond at integer n

μg : microgram

μL : microlitre

μM : micromolar